NATURE

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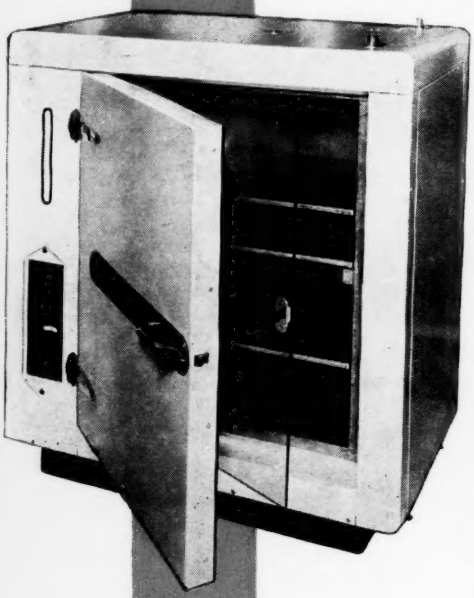

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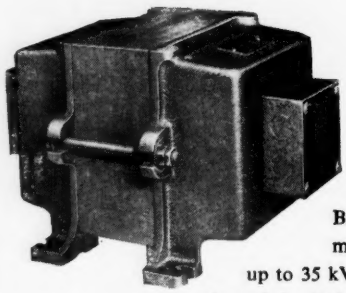

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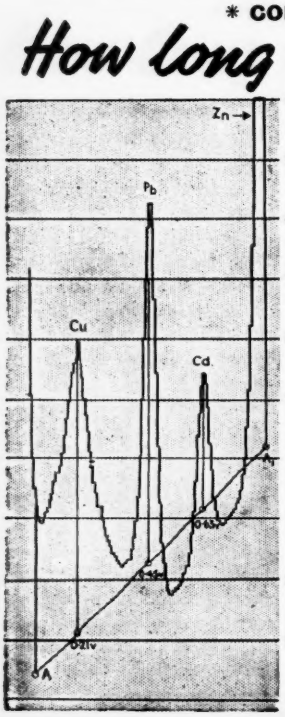

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ORGANIZATION OF SCIENTIFIC AND TECHNOLOGICAL RESEARCH IN BRITAIN

IN the election manifesto of the Conservative Party released on September 11, it was stated that, as the first of five measures to promote technological advance in Britain and to translate this into productive capacity with a high and rising rate of investment, one Cabinet Minister would be given the task of promoting scientific and technological development. "While it would be wrong to concentrate all Government scientific work into a single Ministry, this Minister for Science will have responsibility for the Department of Scientific and Industrial Research, the Medical and Agricultural Research Councils, the Nature Conservancy, the atomic energy programme, and the United Kingdom contribution to space research". The manifesto added that the development of nuclear energy for peaceful purposes would be pressed ahead, a conference called for those concerned in industry and education to forward the spread and understanding of automation, while besides encouraging new inventions and the development of new techniques, further changes would be made from time to time in the functions of Ministers as might be necessary to meet modern needs.

This emphasis on the importance of science in the national welfare and the need to adapt administration to ensure that due account is taken of scientific and technological advance, was followed on September 17 by a special statement from the Labour Party entitled "A New Deal for Science". This statement proposed the appointment as "Minister of Science" of a senior Minister with general responsibility for scientific affairs and the authority to perfect and carry out by and through the various Ministries concerned a carefully planned programme to use modern science to the full. This programme would include a further expansion of scientific and technological education, more scientific training in the schools, and consequently more science teachers, a substantial increase in the number of research and development contracts and Government grants to individual firms for approved long-term research projects; and the establishment under the Minister of a scientific and technical planning board to advise on the direction of industrial research and development, on the awards of research contracts and on the grants to individual firms.

Both major political parties of Britain have thus committed themselves to some modification of the organization of research and development in the country, and of the means by which scientific and technical advice is presented at the highest level. Neither statement gave sufficient information to allow a sound judgment as to which is the more promising proposal, but now that Mr. Macmillan has been returned to power he has lost no time in making the promised appointment. The Cabinet changes announced on October 14 included the appointment of Lord Hailsham as Lord Privy Seal with the general duty of promoting scientific and technological advance in consultation with the departmental Ministers. No Ministry of Science is to be created; indeed, it is pertinent to observe that Lord Hailsham gave last April a reasoned argument against the practicability of any such appointment, and it is clear that Ministers will retain executive responsibility for scientific matters within their own departments and that no attempt will be made to limit the freedom and initiative of universities or industry in determining the content of scientific education or the direction of research.

Lord Hailsham, who relinquishes the chairmanship of the Conservative Party, takes with him, from the Lord Presidency of the Council, ministerial responsibility for the work of the Department of Scientific and Industrial Research, the Medical Research Council, the Agricultural Research Council and the Nature Conservancy; this constitutes a definite break with the structure built up in the past forty years and more. He will also assume responsibility for the work of the Atomic Energy Authority, the Atomic Energy Office, the Overseas Research Council and supervision of the British contribution to space research. At a Press conference on October 14, Lord Hailsham indicated that his task would be to do some fundamental thinking on the relations between Government and science, and he stressed his anxiety to do nothing to interfere with the independence of the Government-financed scientific bodies. Essentially he confirmed the views he expressed last April to the Institution of Chemical Engineers, and indicated that the appointment was only likely to show results after a long period and that its terms were not such as could easily be put down on paper.

It is thus unlikely that Lord Hailsham's appointment contributes anything material at the moment to the formal organization of the scientific effort of Great Britain, either in research and development or in other fields. Lord Hailsham's addresses to the Institution of Chemical Engineers and the Institute of Physics last year showed how well aware he is of the dependence of our scientific and technical effort not merely on the resources allocated for such work, but also on education, and the interlocking of problems of application with those of defence as well as of industry. Equally he recognizes that if educational and scientific institutions are to function efficiently, they must retain complete integrity, and accordingly a high degree of independence. His conception of a scientific policy—and presumably in this he includes technological policy—is of a partnership in which Government, teaching institutions, research institutions and industry all play important and interrelated parts. It could well be that Lord Hailsham was selected for his new office because he has publicly stressed the need for a more intelligent understanding of the forces at work and has indicated that he was

already thinking deeply about the relations between Government and science.

There can be little doubt that Lord Hailsham intends to consider whether the new arrangements are adequate, and whether they can be improved; but there seems little reason to fear that new administrative arrangements will be created without due forethought and consultation, or new policies concerted and applied unimaginatively or without due regard to the essential conditions for scientific work. It may be expected that Lord Hailsham's inquiries and thinking will lead to the elimination of some of the duplication of effort that can be found, though it is improbable that he will fail to recognize that there are occasions when such duplication is an advantage and an asset rather than waste. What is important is that Lord Hailsham appears to be bringing to his task the breadth of vision, the imagination and something of the understanding of the mind of the scientist that are essential for success.

What is more important, perhaps, is that Lord Hailsham himself recognizes that the task of Minister of Science or for Science is inherently impossible for any one man to discharge; and he is likely to look rather for the administrative arrangements which may best permit the Government as a whole to take full account of the scientific and technical factors involved in its decisions on policy, and to ensure an adequate and balanced apportionment of our resources in man-power, in finance and in materials for scientific and technical work generally. If also he examines the question whether, as has been alleged, the authority of scientists in the key Departments of State is really being dangerously eroded, he will have done as much as can fairly be expected of any one man.

The statement, "The First Minister for Science", which Lord Hailsham has since issued (see p. 1263) of this issue of *Nature*) confirms all this. He emphasizes the need for other Ministers in the Government to be scientifically minded if his work is not to be incomplete, and he stresses again that his appointment should impel scientists themselves to take a share in thought about scientific policy. Such a policy, he reiterates, cannot be the product of Government thinking alone.

Several specific points in this statement should also be noted. Lord Hailsham believes that Government science, and perhaps all British science, is at present too parochially minded, too departmentalized and lacking in broad vision. He believes also that the Advisory Council on Scientific Policy holds the key to the situation, and announces his intention of relying more upon this Advisory Council for general advice on questions of scientific policy. With this welcome indication that the Advisory Council's authority is to be restored comes the admission at last that the office of the Lord President of the Council has been under-staffed, and the warm welcome which scientists generally will extend to the whole tenure of Lord Hailsham's statement will be accompanied by greater confidence in that he is now likely to enjoy the services of a larger and more flexible office staff. Lord Hailsham's efforts to tackle such questions as the general balance of scientific effort, to forge closer ties between Government research stations and institutes and the universities, and to foster alike applied research and increased benefactions for universities and colleges, will carry the greater conviction in this context, and it should also assist to enlist the support of scientists and technologists generally, for which he pleads.

Above all, it is important that Lord Hailsham's appointment should be regarded as a challenge to scientists and technologists generally to think deeply about the issues displayed in this statement, many of which were set forth earlier by Lord Hailsham and Sir Hugh Beaver in their addresses to the Institution of Chemical Engineers. It should not be forgotten that, however admirably an administrative structure may be devised and established, its effectiveness will always largely depend on the men who use it. Apart, too, from the perennial temptation to look for rapid political returns rather than the long-term gains which a sound scientific policy would offer, the penetration of science into the more backward industries and into some Departments of State is a matter both of education and of personal influence. Personalities again may play a vital part, especially when it is a matter of securing co-operation.

It is accordingly worth while to look carefully at the way previous arrangements have functioned before deciding on further changes. Valuable clues may well be found as to the essential conditions, and even in the context of the new responsibilities which Mr. Macmillan has entrusted to the office of Lord Privy Seal, Lord Hailsham will not find it easy to give better services to science than have been rendered by some of his predecessors as Lord President of the Council, notably Lord Balfour and Lord Waverley. The late Sir Walter Morley Fletcher has written of Lord Balfour's deep interest in the work of the Medical Research Council, the chairmanship of which he retained when he became Lord President in spite of the anomaly. Lord Balfour was no less interested, however, in the work of the Department of Scientific and Industrial Research, and sought strenuously to increase effective contact between scientists themselves and also between them and the administrative departments, promoting for this purpose the establishment of the Committee of Civil Research, the counterpart of the Committee of Imperial Defence.

Besides the testimony of members of these Councils and Committees to the encouragement and inspiration derived from the experience of encountering a statesman who possessed both the power to help research and the imagination to understand its value to the State, there is on record the further services to science which Lord Balfour rendered at the Imperial Conference of 1926. Balfour said at the time that he regarded the work of the Research Sub-Committee of the latter Conference, over which he presided, as only second in importance to that of the Committee on Inter-Imperial Relations, and in its proposals for co-ordinating research he sought to

forge new cultural links between all parts of the Commonwealth, through the departments and individuals engaged in scientific work in every field. The recently formed Overseas Research Council owes something to the foundations thus laid, and it might well be worth re-examining the circumstances which have held back for a generation such a promising start.

If there is thus on record how much can be achieved by a Minister with vision and imaginative understanding of the needs of the scientist, there is also testimony to the value of a Cabinet Minister himself trained as a scientist. Such tribute has been paid in regard to both Sir Stafford Cripps and Lord Waverley, notably in regard to the latter by Earl Attlee and also by Lord Winster, who during the Second World War had been given responsibility as a Cabinet Minister for the promotion of science and was for a time a member of the Cabinet's scientific advisory committee (out of which the Advisory Council for Scientific Policy later developed) when Lord Waverley was Lord President of the Council. Lord Hailsham himself has stressed the authority which Lord Waverley could derive in scientific matters from being able to speak as a science graduate. Lord Bridges has testified to the outstanding quality of Lord Waverley's work as Lord President of the Council, not only to his capacity for understanding and sound judgment, but also to the way in which he worked so far as possible through departmental staffs without building up any large organization of his own, and also to the importance of the complete confidence which existed between the Lord President and the Prime Minister.

Lord Bridges, in his Royal Society memoir, referred particularly to Lord Waverley's services to science both while he was Lord President during 1940–43 and afterwards, particularly in what might be called the scientific administration of the development of nuclear energy. It is too early yet to expect the critical biography of Lord Waverley which might illuminate these arrangements more fully, but it is important that they should be examined as fully as possible in the light they could throw on the kind of arrangements which are desirable to-day. They suggest, for example, that any constructive thinking should include a careful appraisal of the functioning of both the Advisory Council for Scientific Policy and the Research Defence Policy Committee.

Even this cursory review indicates the critical importance of personal factors, and there is a further reason for looking closely into this aspect. The late Sir Henry Tizard, who has claims to be considered as a military scientific genius, was very successful in his joint chairmanship of the Advisory Council for Scientific Policy and the Research Defence Policy Committee during 1946–52. Nevertheless, in spite of the conspicuous services he had rendered even before the Second World War in developing defence research policy and later in encouraging the development of operational research, in 1942 he suddenly resigned from most of his official appointments to become president of Magdalen College, Oxford. Sir Henry was one of the easiest of colleagues, so any administrative structure which led to such a decision is *ipso facto* suspect. Incidentally, in their Romanes and Haldane Memorial Lectures, respectively, both Lord Waverley and Sir Henry Tizard have recorded considered opinions and constructive proposals for providing the Government with more authoritative guidance in technical matters.

It may be hoped that Lord Birkenhead's official biography of Lord Cherwell will in due course throw some light on these matters. Sir George Thomson in his Royal Society memoir refers briefly to the existence of conflicts of opinion between Cherwell and Tizard which were well known in the scientific world, but no light is thrown on this by Sir Roy Harrod's more recent personal memoir, "The Prof.". He does, however, indicate another aspect of some interest in considering the functions of a Minister of Science. It is the personal relations between Lord Cherwell and Sir Winston Churchill, first as First Lord of the Admiralty and later as Prime Minister, rather than Lord Cherwell's own subsequent position in the Cabinet as Paymaster-General that are of primary interest. Lord Cherwell was able to render his great contribution to the national war effort because of the extent to which he possessed Mr. Churchill's confidence and understanding.

What Sir Roy Harrod writes of this relationship will bear pondering. Where such confidence and understanding can be established, from whatever beginning, the outcome may well be as effective for the promotion of science and technology as when the Cabinet Minister concerned has himself been trained in science. It might possibly be a waste of scarce and highly trained man-power, even if it were possible, to provide every Cabinet Minister carrying departmental responsibilities involving highly technical and scientific matters or research with a scientific adviser of Cherwell's quality for his personal assistant. The results in any event would depend alike on the quality of that adviser's mind and on the personality of the Minister, as well as on the intimacy of the relations between him and his adviser; but it should be clear enough that it is not sufficient for scientific and technical advice to be presented at one point alone. The effective formulation of policy where science and technology are concerned will come when such factors are accurately assessed, not by one Minister only, but all the Ministers concerned.

There could well be found here the reason why the single-handed efforts of Lord Balfour a generation ago and the advocacy of such men as Lord Samuel have hitherto failed of full fruition. For that reason alone the functioning of the advisory committee compared with that of the personal assistant or adviser requires further examination. Moreover, in Sir Roy Harrod's memoir of Lord Cherwell the emphasis is on the contribution which Lord Cherwell and his statistical section made to quantitative thinking about the conduct of the War, and Sir George Thomson's memoir appears to confirm that this was the most important characteristic of Lord Cherwell's contribution. This again suggests that there is something to be learned from the functioning

of this section and the work of the Central Statistical Office established in 1941.

There can be no doubt as to the immensity of the task which Lord Hailsham has undertaken, and the diversity of problems he will need to consider. Now that he has divested himself of his party political responsibilities as chairman of the Conservative party, he appears to be as good a choice as could have been made. His experience as Lord President of the Council has already given him a close insight into some of the problems involved and his speeches have shown both that he is thinking deeply about the real issues and that he is averse to hasty and unilateral decisions.

If it should be reiterated that Lord Hailsham's appointment represents no essentially new departure, similar positions having been occupied by Lord Balfour more than thirty years ago and by Lord Winster and possibly Lord Waverley in the Second World War, the scale and range of problems now confronting him cannot be compared with those presented to any predecessor in peace-time. He will doubtless examine the functioning of the Advisory Council for Scientific Policy and its interrelations with the Defence Research Policy Committee, as well as the specific problems and relations of the several research councils and organizations for which he is specifically responsible. There is the question of departmental research as against centralized research, for example, under the Council for Scientific and Industrial Research. There is a whole range of problems presented by the industrial research associations and the stimulation of research and its exploitation in industry. There is the matter of the balance of our research effort generally and the place of the universities and the independent research institutions and the resources at their disposal and the way in which Government policy can best strengthen such resources without infringing academic or professional autonomy. There are problems which will lead Lord Hailsham into the educational field, as when he considers our resources in scientific and technical man-power and their expansion, as well as their most effective deployment.

Nor are all these problems sharply separated. The support, for example, which the Department of Industrial and Scientific Research already gives to the universities, both by way of grants to individuals for postgraduate study and to university departments in support of specific research projects, already takes it within the orbit of the University Grants Committee, and this holds in varying degree for the other research councils also. It is arguable that nowhere below the level of the Prime Minister himself can all the responsibilities involved rest in one man, if indeed the task is not too immense for any person to discharge it alone. There, of course, we touch the question of Cabinet responsibility, but without entering on any such discussion it might be observed that the Cherwell–Churchill relation seems at least to support the view that a Minister who carried general responsibility for scientific (and presumably also technological) matters might be able to give the Cabinet sufficient detailed advice to clarify and facilitate decisions either in the Cabinet or by the Prime Minister himself. Lord Hailsham is obviously approaching these questions without preconceived ideas as to whether changes are required in our present arrangements, and if so, what changes for the better are possible, and he has at his disposal much valuable evidence not merely from Lord Waverley, Lord Bridges and Sir Henry Tizard, but also from others like Lord Hankey, Lord Ismay and Sir Henry Dale. Whatever the administrative structure may be, there is one essential condition if it is to function smoothly: effective measures must be taken to eliminate or bridge the gap between what Sir Charles Snow has described so vividly as the two cultures. The effective use of science and technology for the public welfare and in the affairs of State will not be ensured by establishing a Ministry of Science or appointing a special Minister. It will be secured in the measure, and only in that measure, in which Ministers and administrators, the Departments of State, the public corporations, industry and the public generally are aware of the conditions and nature of scientific work, understand in some degree what science is doing and are prepared to provide the necessary support.

THE PRINCIPLES AND PRACTICE OF CROP PROTECTION

The Scientific Principles of Crop Protection

By Hubert Martin. Fourth edition. Pp. viii + 359. (London: Edward Arnold (Publishers), Ltd., 1959.) 65*s*. net.

Advances in Pest Control Research

Vol. 2. Edited by R. L. Metcalfe. Pp. vii + 426. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1958.) 94*s*.

Recognition of Diseases and Pests of Farm Crops

By Ernst Gram, Prosper Bovien and Chr. Stapel. (Danish Agricultural Information and Advisory Aids Service.) Pp. 128 + 112 colour plates. (Cambridge: W. Heffer and Sons, Ltd., 1958.) 35*s*. net.

The Control of Pests and Diseases in Agricultural and Horticultural Crops

By G. L. Hey and K. Marshall. (Agricultural and Horticultural Students Series.) Pp. 172. (London: Vinton and Co., Ltd., 1958.) 12*s*. 6*d*. net.

TO say that, nuclear wars apart, the greatest problem of the future will be to feed the rapidly increasing human population is none the less true for being trite. Happily there is no need to think it is insoluble. Average crop yields are so low that the scope for improvement is enormous, and starvation can be avoided for a long time simply by improving the health of crops. Over much of the world most crops are left to fend for themselves, unaided in their struggle with pests and diseases. What annual toll these predators take cannot be estimated at all accurately, but there is little doubt that human beings will have at least twice as much to eat when they stop sharing their crops with pests and diseases.

The four books listed above differ greatly in content and immediate purpose, but all have the same ultimate aim of increasing yields by protecting plants against

their pests and diseases. They make it clear that many of the losses now suffered are legitimately described as avoidable, in the sense that they could be avoided by applying existing knowledge more widely, but it is also clear that much new knowledge will be needed before all losses come into this category. Between them the books also show how complex and varied are the problems entailed in improving the health of crops. First, there are the research problems, diagnosis of the cause, the biological study of the pest or pathogen to find where it comes from, how it spreads and whether there is a stage in its life history at which it is especially open to attack, and this to be followed by tests of pesticides, of variations in cultural practices or of seemingly resistant varieties. Secondly, when research has produced a control method, there still remains what is often the more difficult task of getting it established in practice, and even with this achieved the position must be closely watched to ensure that the method remains effective and has no undesirable side effects.

"The Scientific Principles of Crop Protection" is one of the few books that attempt to cover the whole subject, and a new edition was long over-due, for most of the insecticides currently used have been discovered since the third edition was published in 1940. The title has been changed to the extent that 'plant protection' has become 'crop protection', but the presentation and approach are as before. The new edition might have been even more welcome had the changes been greater, for it is now more difficult than twenty years ago to contain the subject in a single volume and some parts get scant attention considering their recent growth. Whether the parts briefly mentioned should have been omitted in favour of more detail on those in which the author is most authoritative, however, is a moot point, for in these days of increasingly narrow specialization it is certainly refreshing to find someone willing to look at the subject broadly, even though the looks in some directions may be only glances.

Review journals are necessarily complements to standard texts. They are better able to keep up with advances in the subject and they provide opportunities for specialists to present detail inappropriate in a general book. The eight articles in volume 2 of "Advances in Pest Control Research" cover a wide range of subjects and some authors have undoubtedly made full use of the opportunity to be detailed. Here the contents can only be indicated by summarizing the titles and naming the authors: fluid kinetics of sprays (R. P. Fraser, 106 pp.), toxicity of fungicides (S. E. A. McCallan and L. P. Miller, 28 pp.), seed and soil treatments with insecticides (H. T. Reynolds, 48 pp.), the use of isotopes to measure spray residues (C. T. Redeman and R. W. Meikle, 24 pp.), wool digestion and moth proofing (D. F. Waterhouse, 56 pp.), the relation of chemical structure to herbicide activity (R. L. Wain, 44 pp.), chemical structure and activity of DDT analogues (R. Riemschneider, 44 pp.), the spread of resistance to insecticides in pests (A. W. A. Brown, 64 pp.). It is to the credit of the authors that most have accepted the editor's invitation to evaluate their subjects critically and have not simply summarized published work.

The other two books are for the grower of crops rather than the research worker. "Recognition of Diseases and Pests of Farm Crops" is a most welcome and most unusual book. The text consists simply of captions to the 112 magnificent colour plates containing more than 700 beautiful water-colour paintings by I. Frederiksen and E. Olsen. The sole purpose is to aid diagnosis, which the book will do permanently and everywhere, and methods for controlling the pests and diseases so accurately portrayed are deliberately omitted because they often change and may differ in different countries. The book by G. L. Hey and K. Marshall complements the work of art from Denmark and contrasts strikingly with it. It is most valuable for its succinct recommendations for controlling specific pests and diseases, is least satisfactory for diagnosis, and its pictures are neither beautiful nor very helpful. With both books, the farmer or gardener is well equipped to avoid much loss, and it is no mean testimonial to the achievements of plant pathologists that growers now have reliable methods to control so many pests and diseases.

F. C. Bawden

FREE RADICALS

Free Radicals as Studied by Electron Spin Resonance
By Dr. D. J. E. Ingram. Pp. ix+274. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1958.) 50*s*.; 9.50 dollars.

Free Radicals
An Introduction. By A. F. Trotman-Dickenson. (Methuen's Monographs on Chemical Subjects.) Pp. viii+142. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1959.) 12*s*. 6*d*. net.

MOST free atoms are associated with one or more unpaired electrons, and the formation of compounds generally involves the complete pairing of such electrons in molecular orbitals. Nevertheless, many molecules, of widely varying degrees of structural complexity, have a single unpaired electron. These bodies, known as monoradicals, comprise the most important group of free radicals, and it is with them that the greater part of both of these books is concerned.

The unpaired electron confers two important properties on the monoradical. In the first place the electron spin is associated with a magnetic moment, which may align itself in one of two ways with respect to an applied magnetic field. Transitions between the energy-levels corresponding with these two alignments can be made to fall conveniently in the microwave region, giving the phenomenon known as electron spin resonance. The transition will be associated with hyperfine structure resulting from the interaction of the electron spin with the various nuclear spins, if any, in the radical. This hyperfine structure may then be used to 'locate' the electron within the molecule, and hence to obtain information about the orbital, and about the structure of the molecule. In the second place, the presence of an unpaired spin within a molecule makes it more reactive, and free radicals often play important parts in chemical reactions, since their reactions can provide convenient paths by which the final products may be produced most easily. Dr. Ingram's book is concerned with the first of these aspects of free radical behaviour, and Dr. Trotman-Dickenson's with the second, in particular with the elementary reactions of free radicals, while avoiding chain reactions and oxidation processes.

The overlap between the books is very small, and it is a pity that they have effectively the same title, differing only in subtitle. In both cases the contents are excellent, and may be recommended to all workers in the field of free radicals.

Dr. Ingram's work falls into two main sections, the first being concerned with the techniques of electron resonance, and with those parts of the theoretical background necessary for the interpretation of the spectra of free radicals. The 'experimental' part will be a most valuable guide to those contemplating work in this field, to many of whom microwave methods will be unfamiliar. The various types of spectrometer are clearly explained, and their relative merits discussed : the appendix, dealing with sources of equipment, is a worthwhile addition. A pleasing account of the sources of hyperfine structure follows. The rest of the book is a sectionalized review, with useful explanations, of work up to the present. There are chapters on stable free radicals (mostly fairly elaborate organic molecules or ions), on 'trapped' radicals produced by irradiation of solids or materials in glasses, and on the radicals formed in the course of polymerization and of pyrolysis (of solids). All are interesting, and suggest future applications. Dr. Ingram's field defines itself well without the rather odd definition of a free radical which he gives (p. 2), in which reference is made to 'normal chemical bonding' ; all free radicals are held together by normal chemical bonding.

The book by Trotman-Dickenson is, in effect, an extended and intelligently written review covering a field of chemistry with a much longer history. It is of great value in so far as it ignores the artificial boundaries which appear to have arisen in chemistry, and because of its systematized account and assessment of a very large amount of material. It is not the author's fault if the available matter for some of the sections is inconclusive. The book will prove useful to those connected in any way with this amorphous field.

Both books attempt short treatments of 'biradicals', from very different points of view. This topic holds much for the future. T. M. Sugden

THE IMPORTANCE OF NEW DETECTORS

Čerenkov Radiation and Its Applications

By Dr. J. V. Jelley. Pp. x+304. (London and New York : Pergamon Press, 1958.) 65*s*. net.

IT is seldom that the first book to appear on any topic is so thorough as this work by Dr. Jelley. The treatment of the largely new subject of Čerenkov radiation is very complete in its scope and the many workers in the field, whether they are engaged in fundamental studies of this form of radiation or in some relatively straightforward application of a tool based on the phenomenon, owe a debt of gratitude to the author for this comprehensive study. Those who heard the evening discourse by Academician Tamm at Geneva in 1958 were given a clear picture of the rather intriguing history of the subject and the relatively sudden introduction of practical detectors, based on the Čerenkov effect, seemed the more surprising.

Jelley has gleaned his material from many rather scattered sources and presented it here in a well-integrated form. The rather extensive theory is well supported by his clear expositions of the experimental work in the field and in this he shows the skill of one who has personally made considerable contributions to the subject. The author makes it obvious that the subject is likely to produce more surprises in the future and those interested will find much to stimulate their thought in this volume. Many fruitful researches can be expected in this field and Jelley himself indicates some of these. Modern physics perhaps owes its rapid progress more to the introduction of novel detectors than to any other successes. We need but mention the Geiger and proportional counters, the Wilson cloud-chamber, the nuclear emulsion-plate, the scintillation counter, the Čerenkov detector and the bubble chamber to realize the fundamental nature of the contribution that stems from the study and perfection of these devices. Moreover, their value extends to many fields beyond that of pure physics.

The volume can be very highly commended as a most readable, careful and thoroughly up-to-date account of the subject.

THE VERY COLD WORLD

Experimental Techniques in Low-Temperature Physics

By Guy Kendall White. (Monographs on the Physics and Chemistry of Materials.) Pp. viii+328. (Oxford : Clarendon Press ; London : Oxford University Press, 1959.) 45*s*. net.

ABOUT a quarter of a century ago, all low-temperature physicists (and there were not many of them) knew each other, and picked each others' brains fairly regularly, so that the dissemination of cryogenic techniques was rapid and satisfactory. To-day, however, when every well-equipped physics laboratory has or should have its cryogenic facility, the older method of communication is no longer adequate. Many physicists all over the world now want to be able to handle cryogenic liquids safely and efficiently ; hence the timely appearance of this useful book on cryogenics, the first for nearly twenty years and the first in the English language.

A wholly satisfactory book on techniques is a difficult thing to achieve, probably because a technique is part of a craft and the communication of a craft is not easily done by the printed word. In spite of this difficulty, here is a book which will be of very great assistance to new hands at the low-temperature game, and of not inconsiderable value to the old hands as well. An especially good feature is the 'feel' it gives for cryogenic physics, which will be appreciated by its practitioners.

There are faults, of course. The book strays too frequently into theory. No one is seriously going to read this book to find out how electron spins align themselves in an external magnetic field, or to study the formal derivation of the absolute thermodynamic scale of temperatures. But there are excellent chapters on heat exchangers and their calculation, on mechanical thermal contact, and on thermometry. The important methods of cryostat temperature control are well described, although it would have been valuable to have included some of the electronic circuit diagrams with magnitudes of quantities to give any desired sensitivity of control.

The chapter on vacuum techniques, soldering and sealing is good so far as it goes, but there is no mention of how to make a soft soldered joint that (*a*) will be mechanically strong, (*b*) will never leak, and (*c*) can be non-destructively unsoldered. Further, there is no mention of leak-hunting, that grimmest of pursuits, or of real or virtual leaks, or indeed of whether or

not the author personally believes in such things as low-temperature leaks. The diagrams of actual apparatus are often too schematic to be of great use; for example, the transfer tube and valve on pages 53 and 55, of the design of both of which I disapprove. There is a mention of the use of spontaneous oscillations as a means of liquid level indication, but no description of this odd phenomenon nor any warning of its often annoying and occasionally horrifying effects.

All of those are, however, minor criticisms. The book contains a mass of useful cryogenic information. Generally it gives highly commendable critical comparisons of various experimental methods, such as those used in calorimetry, and for the first time collects really valuable critical data on emissivities and on total thermal conduction and expansion coefficients between room temperature and 1° K.

J. F. Allen

A BIOCHEMIST'S GUIDE TO THE NERVOUS SYSTEM

Biochemistry and the Central Nervous System

By Prof. Henry McIlwain. Second edition. Pp. vii+288. (London: J. and A. Churchill, Ltd., 1959.) 45*s*.

IT is a considerable achievement for Prof. McIlwain to have produced a second edition of his book (already translated into Spanish and Japanese) in such a relatively short time. The need for a new edition is some indication of the rapid increase in our knowledge of the biochemistry of the nervous system to which the author and his colleagues at the Maudsley Hospital have made valuable contributions.

The layout of this book follows the previous pattern though there have been extensive revisions and additions. There is, for example, a new chapter, which is very much to the point, on the relation of the brain to the body as a whole, and the section on brain lipids has been considerably modified in the light of recent observations. Knowledge of the metabolism and functions of proteins in the brain is still somewhat limited, but until Table 6, on the chemical nature and diversity of brain proteins, can be extended it is likely that progress on this particular aspect will be slow. I was again impressed by details given about the rates of chemical reactions in brain and their relation to the speed of cerebral processes, information which is becoming more valuable as it becomes more extensive.

Much useful information is summarized in diagrams and tables (Fig. 34 and Table 28 are instances of this). It is perhaps significant that one of the column headings in Tables 8 and 9 has been changed from "acid-labile phosphate of adenosine triphosphate" to "acid-labile phosphate of purine nucleotides" in the second edition, thus indicating that the free nucleotide content of cerebral tissues is more complex than had previously been indicated.

The chapter on chemical factors in nervous transmission is an extremely able résumé of a mass of information, and some indication of the speed at which this subject is developing can be deduced from the fact that references to the possible role of γ-aminobutyric acid as a pharmacologically active agent are all dated 1955 or later. Biochemical aspects of the action of depressants and excitants receive their due, and in view of the current multiplication of drugs this summary is of some considerable value. Perhaps the one surprising omission in the book is an appraisal of the extensive work of Geiger and his colleagues on the metabolism of the isolated, perfused brain *in situ*.

Elsewhere the author of this volume has remarked, "Until the central problems of neurochemistry have been successfully tackled and we see more clearly how the nervous system utilizes its large energy supply in nervous transmission and in maintaining the system in a state of readiness to react, and how the brain is moulded to an animal's experience, chemical aspects of most of the neurological sciences—and above all, material approaches to nervous, mental and emotional diseases—remain as empirical as was organic chemistry before the advent of structural formulæ". If this book promotes further studies on this intriguing but complex subject then we shall all have been well served. Indeed, it can be recommended to anyone who wishes to learn something of the biochemical processes underlying nervous activity and the relevance of these processes to a study of mental diseases. A comprehensive bibliography at the end of each chapter and, for a book of its size, a monumental index, make it an extremely useful handbook in a field where suitable handmaidens are hard to come by.

G. B. Ansell

FAUNA OF NETHERLANDS NEW GUINEA

The Animal World of Netherlands New Guinea

By Dr. L. D. Brongersma. Pp. 71. (Groningen: J. B. Wolters, 1958.) n.p.

THE island of New Guinea remains little known to the majority even to-day, and its fauna has received little attention through television or in popular published work. It is one of the remaining major areas in which new zoological discoveries are likely to be made and is a region as yet little touched by commercial development. Its animals, despite their affinities with Australia, are unique and much work remains to be done before they are fully surveyed. Geographically, the island and its outliers form the most western extension of the Sahul shelf, and the study of the fauna is vital to the zoogeography of Australasia. For these reasons the publication of a semi-popular account of the fauna of New Guinea is both timely and welcome. Dr. L. D. Brongersma has produced an interesting and factual book, based on his radio talks on the subject. Written for the layman, the book is concerned with the novel and unusual, but also contains much of interest to the specialist. Notable peculiarities among mammals, birds, reptiles, amphibians, fish and crustaceans are described, with notes on their habits, ecology, economic value and sometimes history. The text is enlivened by field observations, line drawings and photographs. The author stresses the need for nature conservation in New Guinea, with emphasis on the dangers of uncontrolled commercial development and ill-considered introduction. A short chapter and a map of the Sunda and Sahul shelves give a background to the origin of the fauna, and the book concludes with a useful account of the zoological exploration of New Guinea. The author, whose aim was to encourage interest in the fauna of New Guinea and its conservation, has written a thoroughly readable account of the novelties and notable animals of the island.

J. E. Hill

Mineralogy and Geology of Radioactive Raw Materials

By Prof. E. Wm. Heinrich. Pp. xiv+654. (London: McGraw-Hill Publishing Company, Ltd., 1958.) 112*s*. 6*d*.

In recent years more geologists have been engaged in explorations for radioactive ores than in any other hard-rock phase of the mineral industry, and since the lifting of security restrictions many thousands of research papers on radiogeology have seen the light of day. Prof. Heinrich has set himself the task of evaluating and summarizing this vast literature, and the result of his labours is the most outstanding monograph on uranium and thorium mineralization that has yet appeared in any language. The work begins with approximately 150 pages on systematic mineralogy, continues for 400 pages with a lucid and descriptive classification of the world's radioactive ore deposits, and ends with 100 pages of bibliography (1,000 items) and comprehensive indexes. Not all his views are uncontroversial: for example, the designation of the Witwatersrand and Blind River ore-fields as respectively mesothermal and hypothermal epigenetic mineralizations will find little acceptance in the placerist schools of South Africa and Germany. But all geologists concerned with the radioactive elements, academic workers and prospectors alike, will find interest and inspiration in these pages. Nearly all the 200 text-figures are new to text-book literature. Some minor errors in place-names, in the failure to recognize synonymous localities (for example, Kasolo and Shinkolobwe) and in the consistent mis-spelling of the mineral names thucholite and parsonsite should be corrected in the second edition, which will doubtless be required as soon as the present glut of uranium is taken up by industry and geologists are once again called on to find new ore fields.

C. F. Davidson

Substitution at Elements other than Carbon

By C. K. Ingold. (The Fifth Weizmann Memorial Lecture Series, May 1958.) Pp. viii+52. (Jerusalem: The Weizmann Science Press of Israel, 1959. Distributed by the Weizmann Institute of Science, Rehovot.) n.p.

This little book is a record of four lectures and is divided into two chapters. The first of these discusses substitution in some co-ordination compounds; principally derivatives of cobalt (III). Attention is directed to the stereochemical course of substitution by the unimolecular and bimolecular mechanisms, and to the particular problems associated with group replacements in molecules of octahedral symmetry. An appendix is also provided to support further the theory of a direct S_N2 mechanism.

The second chapter is concerned with nitration and nitrosation at nitrogen and oxygen atoms. There is much more evidence available in this field and the author has surveyed it lucidly and succinctly. The various reagents and mechanistic ambiguities are well set out and some ingenious experiments which exclude many of these alternatives are carefully detailed.

The work is clearly printed and the diagrams are well produced. There is no index, but a generous bibliography at the end of each chapter which is of greater value in a book of this length. The only error I noticed is the omission of the minus sign from NO_3^- in the last relevant equation on page 32.

This book is valuable both as a summary of the present position in this rather neglected study and for the outlining "of a mechanistic pattern", to quote the author's own words. In his preface Sir Christopher suggests that prospects are extensive, and I feel that the analysis given in this work signposts the way well into a largely uncharted region.

R. I. Reed

Contact Catalysis

By Dr. R. H. Griffith and J. D. F. Marsh. Pp. x+300. (London: Oxford University Press, 1957.) 50*s*. net.

In these days of many-volume treatises on catalysis, the smaller book also has its place; and the publication of a third edition of Dr. Griffith's well-known text-book on contact catalysis, now written with the collaboration of J. D. F. Marsh, is both timely and welcome. It continues to give a condensed and well-planned survey of many of the more important classes of reactions involved in heterogeneous catalysis, together with an adequate treatment of the underlying theory.

In order to accommodate, in a condensed form, the large amount of additional material which has resulted from the intense study which this subject is receiving, three new chapters have been added. These deal, respectively, with modern practice in the preparation and empirical evaluation of stable catalysts having a high specific surface, with the part played by electronic factors in determining the catalytic activity of metals, and with a number of reactions involving hydrocarbons. There has also been considerable rearrangement of the older sections of the book, and much of the descriptive matter on the promotion of catalysts by small amounts of subsidiary components and on the poisoning of catalysts has been revised and brought up to date. This applies also to the chapter on the part played by geometrical factors, and especially by interatomic distances, in accommodating without undue strain the reactive portions of catalysable substrates. The volume ends with a critical survey of modern progress in catalysis, with some forecasting of possible future developments. This book continues to form an excellent summary of modern trends in catalysis.

E. B. Maxted

Practical Animal Ecology

By W. H. Dowdeswell. Pp. 316+16 plates. (London: Methuen and Co., Ltd., 1959.) 32*s*. 6*d*. net.

In many British schools and universities lip-service is paid to the importance of field studies in zoology, whereas the actual practical effort is often limited to a few days at a field study centre or attendance at a single Easter-course in marine biology.

Here, almost for the first time, is a book which will really assist teachers and lecturers to introduce their students to modern animal ecology. The book lives up to its title. A wide range of methods and techniques is described which are applicable to the study of terrestrial, freshwater, marine and brackish water habitats. In some cases guidance is given on the construction of what would otherwise be expensive pieces of apparatus. To get the best out of this book, the student should be given considerable help by his teacher. So much is packed into it that many students let loose with it will suffer ecological indigestion.

On modern standards the price is reasonable, for in my opinion this book should have a major effect on the teaching of animal ecology in schools.

J. B. Cragg

THE FIRST MINISTER FOR SCIENCE

By the Right Hon. VISCOUNT HAILSHAM, Q.C., LORD PRIVY SEAL AND MINISTER FOR SCIENCE

THE proposal for a Minister for Science was first talked about when the Conservative Manifesto was being prepared. I was myself at first very sceptical. It seemed to me that there were two very great pitfalls to be avoided. On one hand there was the danger that the scientific world—the Royal Society, the universities, the industrial scientists, the Atomic Energy Authority and the Research Councils—the Department of Scientific and Industrial Research, Medical Research Council and Agricultural Research Council, Overseas Research Council and Nature Conservancy (the governing boards of which are very largely manned by volunteers)—would think that too much was intended, and that the new Minister would interfere with the independence and integrity of the scientist in his own sphere. If that danger were not avoided, I feel that I should lose the confidence of those on whom all else depends and whose co-operation I must win if I am to succeed at all. I hope and believe my appointment has allayed these fears. After all I am the same man who has been Lord President of the Council for the past two years. My general attitude to science and scientists has been made plain to those who were interested, and I believe has won confidence. No change in that general attitude is involved in the new appointment. I have no authority over the universities to compel them to do anything they do not wish to do. My authority over the Atomic Energy Authority and the Research Councils is no greater than my predecessors and I had before, and I have no intention of taking away from them the authority which Parliament has given to them, or the freedom which they now enjoy to perform their functions without detailed interference. I am there to give them general guidance and to help them in their relations with Government, especially other Government departments.

But an equal and opposite danger is to be expected at this point. I can almost hear the cynical comment: "New Presbyter is but Old Priest writ large. The Manifesto promise, the new Minister, is just a piece of political window dressing. Nothing more is to be expected and everything will go on exactly as it was before". I hope and believe this is a mistake, and I would not have accepted the appointment had I believed otherwise.

All the same, I would like to make it plain at the outset that this is a long-range project. You must not expect clutches of satellites to be flung into orbit in a miraculously short space of time. Sponsors of ingenious inventions will be disappointed. The Minister is not a repository of brilliant new thoughts which have failed to appeal to anyone else. He is not an overlord to the Minister of Education and will not therefore be able to create vast new academies of science and technology. He is not "Master of the Queen's Rockets" and will not therefore play a decisive part in the politics of guided missiles. He is not a super Minister of Power nor a Minister of Transport, Agriculture or Health. Indeed, I must emphasize that unless all the other Ministers in the Government—I think without exception—are at least as scientifically minded as I ought to be myself, my work will be frustrated—and until they are my work will be incomplete. I do not think it is necessarily a disadvantage that I am not a professional scientist. The First Lord of the Admiralty is not a naval officer; the Minister of Transport would not necessarily be better for being a railway man. In the main, parliamentary government is government by amateurs advised by experts. The one exception is the Lord Chancellor, and he has a technical legal job to do. In my own case I believe that the appointment of a non-scientist as the first Minister will compel scientists to do the most urgent thing, that is, to share in the thought about scientific policy themselves.

For, whether or not there is need for a Minister or a Ministry, two years experience as Lord President has convinced me that there is need for a policy for science and that policy cannot be the product of Government thinking alone.

In describing my task I will start by one or two general propositions. Science in Great Britain increasingly touches life at every point. It is thus necessarily a partnership—and, since Britain is a democracy, it is necessarily a partnership in which everyone is invited to play a part. But the indispensable partners are industry, agriculture, medicine, teaching, the universities and Government—and, of course, both the scientists and the non-scientists who play their part in the administration of the various branches of public and economic life. In all these spheres my task is to promote science. But only in part of one of these spheres of activity—that of Government—have I the power to achieve this by the use of authority as distinct from encouragement, diplomacy, enthusiasm, example, precept or advice.

Secondly, after two years as Lord President of the Council, I would say that, if only because of the very multiplicity of scientific disciplines, Government science, and perhaps all British science, is at the moment too parochially minded, too departmentalized and lacking in the broader vision. The Research Councils and the Atomic Energy Authority are by their very terms of reference limited in their spheres.

However, a Minister for Science is bound to look for a more generalized philosophy and approach if he is to succeed. In some ways the focus of scientific opinion in Great Britain is the Royal Society. Long may it flourish. But for the purpose of advising the Government on scientific policy, the proper channel already exists in the form of the Advisory Council on Scientific Policy. I believe that this body provides one of the keys to the present situation, composed as it is of a unique connexion of Government and non-Government scientists under an independent chairman with a vice-chairman who happens also to be the chairman of the Defence Research Policy Committee. I shall endeavour to rely more than

ever upon this Advisory Council for generalized advice on questions of scientific policy. My purpose is to make the voice of science coherent and articulate under Government encouragement, and in one real sense to make science self-governing under Government inspiration. For this purpose a greater use of the Advisory Council on Scientific Policy is inevitable. I had already begun to move in this direction in the last Parliament. I hope to go further now, and I feel I am going to be helped by the more flexible and numerous office staff (which supplies the secretariat of the Advisory Council) with which I shall be equipped as the result of the marriage between the Atomic Energy Office and the Lord President's Office. Thus assisted I shall try to tackle such questions as whether the general balance of scientific effort is right or could be better deployed, and other questions of a general nature as they emerge. Already in the last Parliament I had pressed forward an inquiry under the late Sir Claude Gibb (now chaired by Sir Solly Zuckerman) on techniques of management and control of Government financed research and development, and I look forward to a report from them, with positive suggestions, when they have completed their heavy task.

One of the matters I referred to the Advisory Council on Scientific Policy in the last Parliament was the question of space research, and it was as a result of its advice that the Steering Group under Sir Edward Bullard was set up and the group of scientists under Prof. H. S. W. Massey paid their recent and successful visit to the United States. I am not sure that either the philosophy or scope of our policy on space research is yet fully understood, and I would welcome the opportunity of expounding it more fully should questions be directed to me.

I also have great hopes of the Overseas Research Council which I set up at the end of the last Parliament under the chairmanship of Dr. R. S. Aitken. The object of this body is to act as a sort of clearing-house for our research effort overseas both separately and in co-operation with other countries.

I also hope to show a personal interest in the applied research which is very widely carried out by private industry and in industrial research associations under the general authority of the Department of Scientific and Industrial Research. But execution of all this work will continue to rest with the bodies entrusted by Parliament with those functions.

In many ways the clue to the picture of science in Britain lies with the universities—especially with those which have great potentialities for growth and indeed an increase in numbers. To explore this in any detail would be outside my present purpose and indeed outside my function as the Minister for Science. I have no authority over the universities nor over the University Grants Committee, and I have no ambitions to obtain such authority. But I shall try to forge friendly links with both, both personally through individual contacts, and by discussions between officials. In particular, I have ideas for the forging of closer links between the Government research stations and institutes, and the universities, from which I believe both the Government stations (and the scientists who work there) and the universities would stand to gain in prestige and in effectiveness.

I also believe that the time has come for the purse strings of private munificence by industries and industrialists to be opened again for the benefit of universities and colleges and I will do all in my power to produce interest in this. My object here is not to limit the amount of Government help, but to widen the front of public interest, and increase academic independence and flexibility. It would be ungenerous and at this time insensitive not to point in this connexion to the gifts rightly described as princely—including one particularly welcome from the Transport and General Workers' Union, to Churchill College and to the project for St. Catherine's reconstitution at Oxford. But, greatly daring, may I say that I do not see why gifts from individuals and institutions should be concentrated on Oxford and Cambridge or even London ? The greatest scope for enlargement and improvement surely lies elsewhere, as other generous and far-sighted benefactors have recognized.

The teaching of science and mathematics in the schools is again not a matter for me but for the teaching profession, and my contact with that profession must be through the Minister of Education ; but I hope to keep a close liaison with the Minister in this and other matters, and I shall also try to seek guidance from the representatives of independent schools.

It is clear, I think, that in a single term of office only the foundation can be laid of a genuinely scientific approach to the problems of the present day. I hope that my ambitions in this direction will not be despised as inadequate because they are realistic and long-term. My hope will be to engender in all a genuine enthusiasm for science, and respect for scientific work and scientists ; not merely for their practical achievements, but also for the cultural values they represent. If I win their confidence, and also make clear to the public and to my colleagues the nature of their needs and their outlook, I will not have failed. Above all, I would like to say that I bring to this new and creative work all the enthusiasm and desire to serve of which I am capable, and a real determination to see that British science continues to be an instrument of peaceful progress and a means of enhancing British prestige in the world and British prosperity and culture.

ROCKET PROPULSION

At the meeting of the British Association in York, one of the sessions in Section B (Chemistry), on September 8, was devoted to a series of papers and discussions about various aspects of rocket propulsion.

The subject was introduced by Dr. W. B. Littler, director-general of Scientific Research (Munitions), Ministry of Supply, who outlined the history of rocket development. He referred to the early use of gunpowder for rocket propulsion and the introduction in the Second World War of a variety of rocket weapons made possible by the development of new solid and liquid propellants. The German *V*2 rocket, with a range of 200 miles and a payload of 1 ton, was the outstanding technical

triumph of its day. Since the War, vast sums of money have been spent, particularly by the Americans and Russians, on all forms of missile research and development. The all-up weight of some rockets has surpassed the 100-ton level, and a range of 5,000–6,000 miles is claimed for some ballistic missiles. In relation to the resources and man-power employed, some impressive successes have been achieved in the United Kingdom, and important developments have been pioneered with solid and liquid propellants and with the motors associated with them.

The index of propellant performance most frequently quoted is the 'specific impulse'. The solid and liquid propellants now in common use have a specific impulse in the range 180–250, compared with the figure of only 60 for gunpowder. Substantial increases in specific impulse are possible by the use of uncommon fuels and oxidants (for example, hydrogen and fluorine) but most military applications can be met by the use of conventional propellants, and these are adequate for putting artificial satellites into orbit. The most spectacular advances in recent years have arisen from three developments: (1) the substantial increases in the size of the rockets and the weight of propellant carried; (2) the perfection of the multi-stage rocket, whereby motors are discarded successively in flight, after all the propellant has been burnt, thus eliminating dead weight as quickly as possible; (3) the development of very large solid-propellant motors containing up to ten or more tons of propellant.

Unlike liquid-propellant motors, the thrust of solid-propellant motors cannot yet be controlled in flight, but they are basically simpler for many applications and can be more easily maintained in a state of readiness. As a result, their use has greatly extended in recent years, but there is a future role for both types of propellant. For controlled flight in outer space, much higher performances, not attainable with chemical systems, are required.

The first paper, "Thermodynamic Aspects of the Choice of Rocket Propellants", by Mr. G. K. Adams, of the Explosives Research and Development Establishment, Waltham Abbey, discussed the relation between the thermodynamics of propellant ingredients and combustion products and the performance of rocket propulsion systems. Applying the principle of conservation of momentum to a rocket in a force-free field, it can be shown that the velocity increment v is given by: $v = \overline{V} \ln(M_1/M_2)$, where $\overline{V}$ is exhaust velocity of the combustion products, M_1 is initial mass of rocket, and M_2 is mass of rocket after all the propellant has burnt. The exhaust velocity is used as an index of propellant performance. It is generally quoted in the form of the 'specific impulse', which is the quotient of the exhaust velocity and the force-to-mass conversion factor, and has the dimensions of time.

By applying the principle of conservation of energy, the square of the exhaust velocity can be shown to be proportional to the decrease in total enthalpy per unit mass on burning the propellant under rocket conditions. It depends, therefore, on the initial chemical energy of the propellant and on the efficiency with which this is converted into translational energy in the exhaust jet.

The demand for high chemical energy per unit mass suggests the choice of elements of low atomic weight; suitable oxidation-reduction reactions between these have energies in the range of 2–4 kcal./gm. The energies of reactions between free atoms or radicals are much greater, but owing to their high reactivity even at extremely low temperatures, there appears to be little hope of utilizing them in propulsion systems. Other factors in addition to those of energy must also be taken into account. Thus a propellant must have adequate chemical stability, its physical properties must be suitable for the particular application, and the materials used in the construction of the combustion chamber must be able to stand up to the temperatures attained. In practice, such factors tend to limit the range of useful chemical energies still further, and in these circumstances particular attention must be paid to achieving the most efficient conversion of chemical energy into translational energy of the rocket.

The efficiency of the expansion process is governed by a number of considerations. A low total heat capacity per unit volume of gaseous products is beneficial; this leads to a requirement that the gaseous products shall contain the minimum number of constituent atoms (for example, HF rather than H_2O); lower efficiencies result if solid products are formed. Energy released by shifts in chemical equilibria during expansion can be used less efficiently than that released in the chamber. Energy is lost through the non-attainment of velocity and thermal equilibria in systems giving solid products. Chemical energy alone, therefore, is not an adequate criterion for the choice of propellant systems. A change which increases the efficiency is often more useful than a mere increase in chemical energy. Additional factors which have to be taken into account are cost and availability.

Mr. J. E. P. Dunning, director of the Rocket Propulsion Establishment, Westcott, then spoke about "The Application of Liquid Propellants to Rockets". He referred to some of the more important ballistic equations and described the physical processes involved in a rocket engine using a liquid fuel (for example, kerosine) and a liquid oxidant (for example, oxygen). A steady pressure is maintained in the combustion chamber by feeding in the liquids at the same mass flow-rates as the gases are ejected from the nozzle. The propellants are pumped into the head of the combustion chamber through a multiplicity of orifices designed to establish as quickly as possible a uniform mixture of fuel and oxidant. Both liquids must be vaporized and this is brought about by atomization, initiated by the actual process of injection and accelerated by the combustion of preceding droplets. Once established, the combustion process is self-supporting but has, however, many tendencies to instability. Satisfactory geometry of the chamber and injection head is essential to reduce these irregularities to a minimum; but the nature of the problem is such that the empirical approach still has to be largely relied upon.

In the case of a liquid oxygen/kerosine motor developing 100,000 lb. thrust, it is necessary to feed liquid into the chamber at a rate of about 400 lb./sec., of which 280 lb./sec. will be liquid oxygen and 120 lb./sec. will be kerosine. The injection head may have as many as 2,400 orifices each 0·1 in. in diameter, from which the liquids emerge at about 100 ft./sec. Within an axial distance of about 1 ft., and a time of 2–3 millisec., the physical processes of atomization, vaporization and chemical reaction have to take place. In the combustion chamber the temperature attains 3,300° K. and the pressure 500 lb./in.^{2}, and the gases emerge at a velocity of

around 8,000 ft./sec. The temperatures attained are such that the walls of the chamber and the nozzle have to be cooled, and either fuel or the oxidant is used for this purpose. To feed the liquids into the injector head two turbo-pumps are used, since the alternative system of pressurizing the tanks is ruled out by considerations of weight. The pumping power required is large, but is achieved with a propellant consumption of rather less than 2 per cent of that used in the combustion chamber.

In selecting possible fuel/oxidant combinations, performance merits may be over-ridden by criteria such as toxicity, availability and cost. A limit on overall performance may thus be imposed, but this can be countered by increasing the mass ratio (mass at launch/mass at 'all-burnt'), although with a single-stage rocket it is not practicable to exceed a ratio of about 14 to 1. Enhanced performance can then only be achieved by the use of multi-stage propulsion systems.

Dr. G. H. S. Young, of the Explosives Research and Development Establishment, Waltham Abbey, then dealt with British solid propellants for rockets. He pointed out that all solid propellants are explosives and, under appropriate conditions, can be detonated. These conditions must be avoided during manufacture and use, and this consideration frequently limits what can be achieved practically.

The two main solid propellants in use in Britain are extruded cordite, sometimes called double-base propellant, and plastic propellant. The extruded cordites are similar chemically to gun propellants and are available in a wide range of sizes and burning-rates. In general, the burning-rate is adjusted by altering the calorimetric level; the more energetic the composition, the faster it burns. The size of charge which can be produced is limited by the size of the presses available and the hazards involved with large quantities. However, the double-base system has been recently extended by the exploitation of a casting process in which the nitrocellulose is gelatinized in a mould by desensitized nitroglycerine, the charge then being cured at 140° F. for some days. In this way charges larger, and more complex in shape, than those capable of extrusion are being produced. Both extruded cordite and cast double-base are used as loose charges in the rocket motors.

Plastic propellant, however, being a putty-like material, is capable of case-bonding, since the material can accommodate the differential thermal expansion between the motor wall and the propellant itself. This type of propellant has been developed to make use of ammonium perchlorate as the oxidizer. Burning-rates are adjusted by the addition of ammonium picrate and, as with extruded cordite, the lower-energy compositions burn more slowly than those of high energy. This propellant has been successfully used in the largest British solid-propellant rocket to date, namely, that used in the *Skylark* in the International Geophysical Year experiments; this rocket has a charge of about 1,800 lb. of propellant and is 17 in. in external diameter.

Another solid propellant being investigated is the so-called pressed charge, pioneered by Nobel Division of Imperial Chemical Industries, Ltd. In this propellant the ingredients are consolidated to rock-like form by powerful presses. Ammonium nitrate is the oxidizer, and propellants with low rates of burning, particularly suited for assisted take-off units and for gas generators, are produced.

The present trend is for rockets to increase in size, and none of the propellants mentioned, with the possible exception of cast double-base, is altogether suitable for large missiles. Work is therefore proceeding on other castable composite propellants based on synthetic rubbers and, of these, polyurethanes appear to have many advantages. There is also a demand for higher performance and this can only be achieved by the introduction of novel ingredients, such as light metals, or new combustion systems which might, possibly, be based on fluorine compounds. In addition, if solid propellants are to be used in the larger missiles, then methods of thrust control and thrust termination will need to be developed and the reliability of operation will have to be very high.

Finally, Dr. L. R. Shepherd, of the Atomic Energy Research Establishment, Harwell, and chairman of the British Inter-Planetary Society, spoke about propulsion for space travel. The equation mentioned earlier represents an idealized condition and in the actual case of a rocket accelerating from the surface of the Earth the actual velocity may be 1,000–2,000 metres/sec. less than the value predicted by this equation on account of atmospheric resistance and other effects. For even the most modest excursion into space the limitations of conventional propellants demand the use of multi-stage rockets. Using available chemical propulsion systems in staged rockets, it should be possible to put 20-ton payloads into orbit around the Earth or to deposit 1–2 tons of instruments on the Moon. But for any more ambitious mission, propellant systems of much higher performance are required.

Dr. Shepherd considered that the difficulties involved in the application of reactions between free atoms or radicals (mentioned by Mr. Adams) made it seem unlikely that these can be successfully applied to a practical propulsion system.

Another possibility is the utilization of forbidden transitions between excited and ground states in the electron shells of certain atoms, for example, helium. If the active material can be stored and its energy released in the thrust chamber of a rocket engine, the potential usefulness of a small single-stage vehicle is enormously extended. It may be that the storage of active helium at low temperatures is more feasible than that of atomic hydrogen.

Speculations on the application of nuclear power to rocket propulsion generally assume that the energy from a nuclear reactor can be transferred to a suitable working fluid, the optimum material being hydrogen. This might be achieved by heating the working fluid in a thrust chamber and allowing it to expand through a nozzle in a manner similar to conventional practice. Alternatively, the working fluid may be ionized and accelerated as a plasma in a magnetic field. There are many formidable technical problems to be overcome before such nuclear systems can be developed.

The use of electrical methods of propulsion has also been proposed. This would involve an arc discharge to heat a working fluid and expand it through a nozzle, or, to avoid excessive temperatures, the electrical acceleration of a working medium. It is generally assumed that this would be effected by ionizing the working fluid, extracting the positive ions, and accelerating them through an electrostatic field. Alternatively, a fully ionized plasma may be accelerated in an electromagnetic pump.

The working medium of such a system would probably be one of the alkali metals, possibly sodium. At a temperature of 3,500° K. and a pressure of 10^{-6} atmospheres, a sodium plasma is 95 per cent ionized; there should therefore be little difficulty in producing and maintaining such a plasma.

It is known that a great deal of development work on novel propulsion systems is being carried out in the United States and presumably also in the U.S.S.R.

In the discussion, Prof. M. Stacey expressed interest in the possibility of using fluorine in propulsion systems. The high toxicity of the combustion products was cited as a fundamental difficulty, and costs would also be high. In reply to questions concerning the relative reliability of liquid- and solid-propellant motors, it was pointed out that there is very little information to support an absolute comparison. A liquid-propellant motor, being more complex, might be expected to have a greater rate of failure, but the opinion of many British workers is that, given adequate attention in the research and development phases, the reliability of liquid-propellant motors should at least approach that of corresponding solid-propellant motors. Questions were also raised regarding the role of solid 'cigarette burning' charges in view of the desire for high loading densities for propellants. It was explained that the application of this type of charge to larger motors is limited by the need for high burning-rates and the additional insulation necessary to protect the motor wall which is exposed to the hot combustion products as burning proceeds. These factors rob the 'cigarette burning' charge of its immediate attraction.

W. B. LITTLER

DENSITY OF THE UPPER ATMOSPHERE FROM ANALYSIS OF SATELLITE ORBITS: FURTHER RESULTS

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IN an article on this topic in *Nature* some months ago[1], a new method of determining air density from the rate of contraction of satellite orbits was described and applied to the satellites launched during 1957 and 1958. In the present article the method has been refined by taking account of atmospheric rotation, and further results are given, utilizing the satellites of 1959, for heights between 180 km. and 700 km. The variation of density with latitude and season, and day-to-night changes, are also discussed.

The rate of decrease of the orbital period of a satellite, which can readily be measured, depends on the integrated effect of air drag around the orbit. The drag is greatest at perigee, and for a given satellite it is the air density at heights a little above that of perigee which chiefly controls the drag effects and which can best be estimated from the rate of change of period, dT/dt.

Method of Analysis

It is assumed, first, that the drag D acting on a satellite of mean cross-sectional area S, moving with velocity v relative to the centre of the Earth, in air of density ρ, may be expressed in terms of a drag coefficient C_D as:

$$D = \tfrac{1}{2}\rho v^2 F S C_D \qquad (1)$$

where SC_D may be taken as constant, and the factor F is included to allow for the fact that v differs from the velocity V of the satellite relative to the ambient air. F, which is equal to $(V/v)^2$, may be taken as:

$$F = \left(1 - \frac{r_p w}{v_p}\cos i\right)^2 \qquad (2)$$

where r is distance from the centre of the Earth, suffix p denotes perigee, w is angular velocity of the atmosphere (taken equal to that of the Earth) and i is inclination of orbit to equator. For almost all the satellites so far launched, F has been between 0·9 and 1.

The second assumption is that the density ρ at heights above that of perigee may be taken as varying exponentially with height y, so that:

$$\rho = \rho_p \exp\{-(y - y_p)/H\} \qquad (3)$$

where H, which is approximately equal to the scale height, is taken as constant. The value of H is not known accurately at heights above 180 km.; but, if H^* is the best estimate of H, the density at a height $\frac{1}{2}H^*$ above perigee, ρ^*, can be expressed in terms of dT/dt by the equation:

$$\rho^* = -\frac{0{\cdot}158}{\delta}\frac{dT}{dt}\sqrt{\frac{e}{aH^*}}\left\{1 - 2e - \frac{H^*}{8ae} + 0\left(e^2, \frac{H^2}{a^2e^2}\right)\right\} \qquad (4)$$

where $\delta = FSC_D/m$, m is the mass of the satellite, a is the semi-major axis and e the eccentricity of the orbit. If $0{\cdot}02 < e < 0{\cdot}15$ and H^*, the best estimate of H, does not differ from the true value of H by a factor of more than 1·5, the maximum error in the expression (4) for ρ^* is less than 5 per cent. If e is increased to 0·2, the maximum error is 10 per cent. Equation 4 is the same as equation 5 of the previous article, except that SC_D/m has been replaced by FSC_D/m. The introduction of the factor F allowing for atmospheric rotation changes the resulting values of density by 5–10 per cent, and the change is always an increase, since no satellite has yet (September 1959) been launched against the rotation of the Earth.

When $e < 0{\cdot}02$, equation 4 becomes less accurate, and, for $0{\cdot}005 < e < 0{\cdot}02$, can best be replaced by:

$$\rho^* = -\frac{0{\cdot}0644}{\delta a}\frac{dT}{dt}\frac{\exp(ae/H^*)}{I_0(ae/H^*)}\left\{1 + 0(e) + 0\left(\frac{H}{a}\right)\right\} \qquad (5)$$

Table 1. Values of $\delta = FSC_D/m$ for Satellites 1957α–1959ζ

Satellite		Mass m (kgm.)	m/SC_D (kgm./sq.m.)	δ (sq.m./kgm.)
Sputnik 1	1957 α 2	83·6	110	0·0088
Sputnik 1 rocket	1957 α 1	—	63	0·015
Sputnik 2	1957 β	—	59	0·016
Explorer 1	1958 α	14·0	23	0·039
Vanguard 1	1958 β 2	1·47	23	0·040
Explorer 3	1958 γ	14·1	23	0·039
Sputnik 3	1958 δ 2	1,327	207	0·0046
Sputnik 3 rocket	1958 δ 1	—	63	0·015
Explorer 4	1958 ε	17·5	29	0·032
Atlas	1958 ζ	3,960	28	0·032
Vanguard 2	1959 α 1	9·75	20	0·044
Vanguard 2 rocket	1959 α 2	23	18	0·050
Discoverer 2	1959 γ	635	48	0·021
Discoverer 5	1959 ε	635	48	0·021
Discoverer 6	1959 ζ	635	48	0·021

where I_0 is the Bessel function of the first kind with imaginary argument, of order zero.

Evaluation of δ

The main difficulty in applying equations 4 and 5 lies in the evaluation of δ ($= FSC_D/m$), and in particular of SC_D. It is assumed here, as in the previous article, that each satellite rotated about its axis of maximum moment of inertia. For satellites with length/diameter ratio greater than about 2, the extreme modes of rotation are then (*a*) travelling exactly like an aeroplane propeller, and (*b*) tumbling end over end. In (*a*), the axis of spin and the direction of motion are in line; in (*b*), the angle between them is 90°; in practice, the angle may be anywhere between these extremes, and the mean of the values of SC_D for all modes of motion between (*a*) and (*b*) has been taken, the drag coefficient being calculated for free-molecule flow with diffuse reflexion.

For near-cylindrical satellites such as *Explorers* 1, 3 and 4 and *Atlas* (1958 α, γ, ε and ζ), SC_D has been taken as $1{\cdot}85\ \lambda d$, where λ and d are the effective length and diameter, the maximum possible error being 19 per cent. A recent study[2] has shown that a rotating cone of length λ and base diameter d, and of shape similar to *Sputnik* 3 (1958 δ 2), has $SC_D = 1{\cdot}43\ \lambda d$ under mode of rotation (*a*), and $1{\cdot}45\ \lambda d$ under mode (*b*), corresponding to drag coefficients (based on the appropriate mean cross-section) of 2·18 under mode (*a*) and 2·09 under mode (*b*). A drag coefficient C_D of 2·15 based on the mean of the cross-sections under modes (*a*) and (*b*) has been taken here. For the spherical satellites, *Sputnik* 1 and *Vanguards* 1 and 2 (1957 α 2, 1958 β 2 and 1959 α 1), C_D has, as before, been taken as 2·2, based on the mean cross-section, including antennæ. The values of δ for *Sputnik* 2 and the rockets of *Sputniks* 1 and 3 have been obtained by comparison with *Sputniks* 1 and 3, as explained previously[1]. For the *Discoverer* satellites, which are cone-cylinders, δ has been taken as the mean of the cross-sections under modes (*a*) and (*b*), with $C_D = 2{\cdot}2$.

Table 1 lists the values of δ obtained for all the satellites launched before September 1959 whose orbits are known, except *Explorer* 6 (1959 δ), to which the theory is not applicable, since e is greater than 0·2. The values for the *Discoverers* apply during the period after the ejection of the re-entry capsule. If the assumptions already stated are justified, the error (standard deviation) in the tabulated values of δ will probably be rather less than 10 per cent.

Evaluation of Air Density

The air density at height $\frac{1}{2}H^*$ above perigee has been found for each of the satellites listed in Table 1 from equation 4 (or, for *Discoverer* 2, equation 5), the values of H^* chosen being consistent with those given later in this article. For the Russian satellites, values of dT/dt, a and e have been taken from orbital

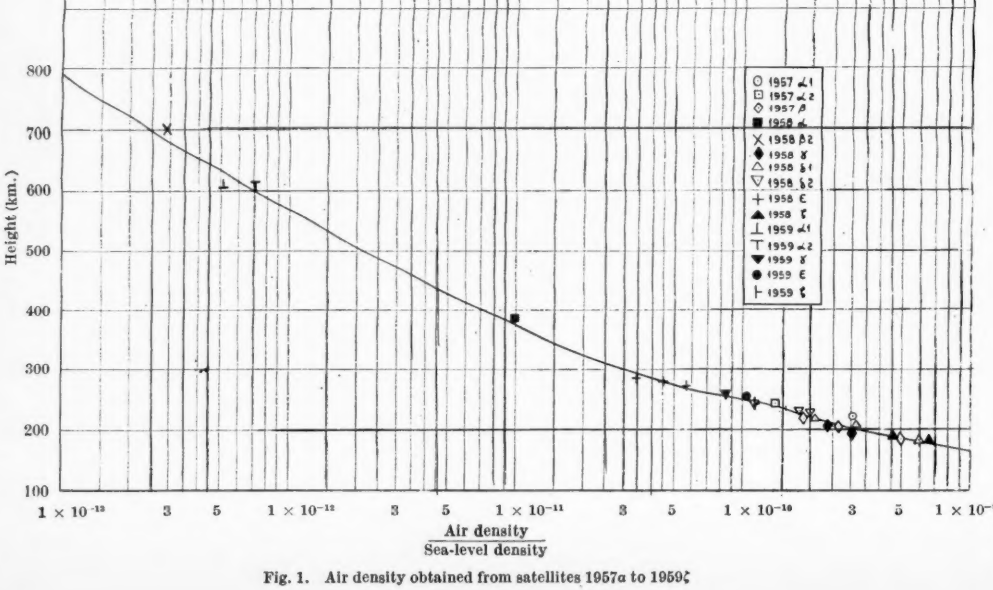

Fig. 1. Air density obtained from satellites 1957α to 1959ζ

determinations made in Britain[3–6]. For the U.S. satellites, values have come from the orbital data issued by the Smithsonian Astrophysical Observatory and by Project Space Track, Bedford, Mass. For satellites with long life-times, the values of dT/dt have been averaged over intervals of several months.

The resulting values of air density are shown in Fig. 1, and a curve has been drawn through the points to represent average density. It is worth noting that the individual points lie close to the curve, except for *Sputnik* 1 rocket, for which, however, the orbital information is rather meagre. None of the other twenty points in the cluster below 300 km. differs from the curve by a factor of more than 1·25. Some scatter is to be expected, because of the errors in δ and because the density varies from week to week by up to 30 per cent at 200 km. height[7] and by 50 per cent or more at 700 km. [8]. Because of the latter variations, and because of the possible influence of charged drag[12,13], the three points above 500 km. are less reliable than those below.

The value of $6{\cdot}7 \times 10^{-15}$ gm./c.c. for air density at 440 km., deduced[14] from the rate of expansion of a cloud of sodium vapour from the Russian Geophysical Rocket of February 1958, differs from the curve of Fig. 1 by a factor of less than 1·25, and fills a gap in a rather empty region.

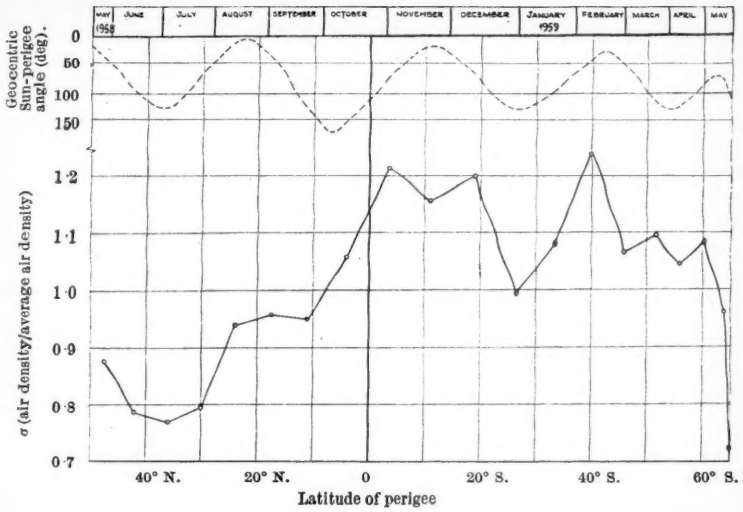

Fig. 2. Air density at the initial perigee height of *Sputnik* 3, 226 km.: variation with latitude and time

Variation of Density with Latitude and Season

The points plotted in Fig. 1 refer to latitudes ranging between 70° N. and 50° S., and to all seasons; but there is no sign of any systematic variation of density with latitude or season. In view of the small scatter, it seems probable that the density at a given height below 300 km. does not depart from its average value by a factor of more than about 1·5 as a result of variations with latitude (between 70° N. and 50° S.) and with season. This is in contrast with the direct measurements from rockets[15,16], which suggest a much wider variation, by a factor of 5 or even 10; so it is worth seeking further evidence.

Such evidence can be obtained from the orbit of *Sputnik* 3 between May 1958 and June 1959. During this time, the perigee latitude moved slowly from 50° N. to 65° S., and the perigee height changed by less than 20 km. The rate of change of period of *Sputnik* 3 thus provides a continuous indication of the air density at heights between 200 and 250 km., and over the range of latitude from 50° N. to 65° S. The air density ρ_p at the current perigee height was calculated from the equation[17]:

$$\rho_p = -\frac{1}{3\delta}\frac{dT}{dt}\sqrt{\frac{2e}{\pi aH}}\left\{1 - 2e - \frac{H}{8ae}\right\} \qquad (6)$$

with $H = 30$ nautical miles (56 km.). The values of dT/dt were obtained from the records of orbital period kept by the Royal Aircraft Establishment (May–October 1958) and the Radio Research Station, Slough (October 1958–June 1959). The density at the initial perigee height y_{po} (226 km.) was then found by multiplying ρ_p by $\exp\{-(y_{po} - y_p)/H\}$, where y_p is the current height of perigee over an oblate Earth. This process gives the air density at the *initial* perigee height (226 km.), but at latitudes near the *current* perigee latitude. The resulting densities, calculated at 20-day intervals and expressed as a multiple σ of the average density, are plotted against perigee latitude in Fig. 2. A satellite encounters drag over a range of latitudes near perigee, and each point in Fig. 2 represents an average density over the 15–20° of latitude within which most of the drag effect occurs, rather than the density at the exact latitude where it is plotted. Consequently, there is no virtue in reducing the time-interval between the points in Fig. 2.

The chief errors in σ are likely to result from errors in dT/dt (estimated standard error 3 per cent), H and $y_{po} - y_p$ (estimated bias errors 20 per cent each) and δ (maximum error[2] 5 per cent). The estimated standard errors in σ from these four sources are 3, $2\frac{1}{2}$, $2\frac{1}{2}$ and $2\frac{1}{2}$ per cent, respectively, implying a standard error in σ of about 5 per cent.

The density at heights of 200–250 km. is known to vary with time, by a factor of up to 1·3, exhibiting a 28-day periodicity, which is attributed to solar disturbances[7–11], and one of the reasons for choosing a 20-day interval in Fig. 2 was to avoid giving prominence to the 28-day oscillations. Fig. 2 represents the combined variation due to solar effects, latitude, season, etc. Since none of the values of σ differs from 1 by a factor of more than 1·4, and the standard error is about 5 per cent, Fig. 2 strongly suggests that the density does not depart from its average value, as a result of seasonal and latitude effects, by a factor of more than 1·5, between latitudes 50° N. and 65° S., thus confirming the evidence of

Fig. 1; it is very unlikely that strong seasonal and latitude variations do occur and happen to have cancelled out for *Sputnik* 3.

It would probably be unwise to draw any positive conclusions about the variation of density with latitude and season from Fig. 2, but the large drop at the right-hand end does encourage the speculation that the air density at heights of 200–250 km. may be low at latitudes south of 60° S., at least in the winter.

Day-to-night Variations in Density

The upper curve in Fig. 2 shows the angle *SCP* subtended at the centre of the Earth (*C*) by the Sun (*S*) and the perigee (*P*) of *Sputnik* 3. If this angle exceeds 90°, the surface of the Earth below perigee is in darkness; if the angle exceeds about 105°, the perigee point itself is in darkness. Comparison of the two curves in Fig. 2, though inevitably inconclusive because of the errors in σ, gives the impression that the air density is related to the angle between Sun and perigee, a possibility which has previously been suggested by Sedov[18], Lidov[19], Groves[20] and others. If so, the air density at heights of 200–250 km. is rather higher on the sunlit half of the Earth than on the dark half, and this day-to-night variation, which had a period of about 90 days for *Sputnik* 3, and an amplitude of perhaps $\pm$ 10 per cent, would be superposed on the 28-day variation due to solar disturbances, which had an amplitude of about $\pm$ 20 per cent. A rather similar interpretation for a height of 700 km. was recently proposed by Wyatt[21], from analysis of the orbit of *Vanguard* 1, though at this greater height the angle between the Sun and perigee is, on Wyatt's interpretation, more important than solar disturbances. There is also some slight indication of a day-to-night variation for *Sputnik* 2 and *Sputnik* 3 rocket, though the 28-day variation is dominant[7,8,11].

Values of *H*

The slope of the density-versus-height curve of Fig. 1 gives the value of the coefficient *H* in equation 3, and Table 2 lists values of density and of *H* derived from the curve. Other curves, of different slopes, could however be drawn in Fig. 1; the individual values of *H* in Table 2 might, therefore, be in error by up to perhaps 20 per cent, though the mean value of *H* between 200 and 400 km. height is almost certainly between 50 and 60 km.

The rather irregular values of *H* in Table 2 between 200 and 260 km. result from the indentation in the curve of Fig. 1. If, instead, a smooth curve were drawn, seven successive points, from six satellites of quite different shape, size and date of launch (*Explorer* 4, *Discoverers* 2, 5 and 6, and *Sputniks* 1 and 3), would lie on the same side of the curve. The indentation, therefore, seems justifiable, though it might still be illusory, if several points happened to be in error in the same sense.

If the indentation is real, it indicates a rather large value of *H*—more than 50 km.—at heights near 220 km., in accord with the values of *H* found from the decrease in the perigee distance of satellites[1,22]. The air temperature depends[1] on the product of *H* and *M*, the mean molecular weight of the air. So, unless *M* varies widely between 210 and 230 km., the indentation corresponds to a peak in temperature near 220 km. height, the maximum value being near 60 *M* °K. The value of *M* is not known exactly, but is probably near 20. Such a peak in temperature might imply the absorption of certain wave-lengths in the solar radiation at heights near 220 km., though this deduction must be regarded as speculative[23].

Table 2. Values of Air Density and *H* given by the Curve of Fig. 1

Height (km.)	Air density / Sea-level density	Density (gm./c.c.)	*H* (km.)
180	$6{\cdot}4 \times 10^{-10}$	$7{\cdot}8 \times 10^{-13}$	27
200	$3{\cdot}2 \times 10^{-10}$	$3{\cdot}9 \times 10^{-13}$	35
220	$2{\cdot}0 \times 10^{-10}$	$2{\cdot}5 \times 10^{-13}$	52
240	$1{\cdot}4 \times 10^{-10}$	$1{\cdot}7 \times 10^{-13}$	46
260	$7{\cdot}7 \times 10^{-11}$	$9{\cdot}4 \times 10^{-14}$	32
280	$4{\cdot}5 \times 10^{-11}$	$5{\cdot}5 \times 10^{-14}$	47
300	$3{\cdot}0 \times 10^{-11}$	$3{\cdot}7 \times 10^{-14}$	57
320	$2{\cdot}1 \times 10^{-11}$	$2{\cdot}6 \times 10^{-14}$	62
340	$1{\cdot}6 \times 10^{-11}$	$2{\cdot}0 \times 10^{-14}$	68
360	$1{\cdot}2 \times 10^{-11}$	$1{\cdot}5 \times 10^{-14}$	75
380	$9{\cdot}3 \times 10^{-12}$	$1{\cdot}1 \times 10^{-14}$	77
400	$7{\cdot}2 \times 10^{-12}$	$8{\cdot}8 \times 10^{-15}$	79
500	$2{\cdot}1 \times 10^{-12}$	$2{\cdot}6 \times 10^{-15}$	86
600	$7{\cdot}1 \times 10^{-13}$	$8{\cdot}7 \times 10^{-16}$	90
700	$2{\cdot}5 \times 10^{-13}$	$3{\cdot}1 \times 10^{-16}$	99

Conclusions

A consistent picture of the air density at heights between 180 and 700 km. is obtained from the orbits of 15 satellites (see Fig. 1 and Table 2). The picture is more complete and more reliable at heights below 500 km. than above. All the results refer, however, to the years 1957–59, and it is probable that the density varies in the course of a sunspot cycle. There is some indication of a peak in temperature near 220 km. height, but there is no sign that density varies with latitude or season by a factor of more than 1·5. Analysis of the motion of *Sputnik* 3 confirms this latter conclusion, for a height of 220 km., and shows some evidence of day-to-night variation in density.

I wish to thank Mrs. D. M. C. Walker for preparing the diagrams and tables in this article.

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NEWS and VIEWS

Nobel Prize for Chemistry for 1959: Prof. Jaroslav Heyrovsky

PROF. JAROSLAV HEYROVSKY, director of the Polarographic Research Institute of the Czechoslovak Academy of Sciences in Prague, has been awarded the Nobel Prize for Chemistry for 1959, for his discovery and development of polarography. A native of Prague, he studied under Sir William Ramsay and F. G. Donnan in London before the First World War and then returned to Prague to continue his research work. It is of interest that the work on the determination of the electrode potential of aluminium which led eventually to the development of the polarographic method was suggested to him by Donnan. The first polarographic apparatus was made in 1925, but the method did not become widely recognized for a further decade, and Heyrovsky's major book, "Polarographie", did not appear until 1941. However, the number of papers dealing with polarography now approaches the 10,000 mark, and the technique finds application in many fields of chemistry and biochemistry; it has had a profound influence on analytical chemistry, for some determinations which are difficult or impossible to carry out by other means yield readily to polarographic treatment. In addition, there have been made non-analytical applications. For example, the kinetics of electrode reactions and of chemical reactions associated with redox processes have been studied, redox potentials have been determined and the energetics of the reduction of organic compounds have been elucidated. Prof. Heyrovsky has not enjoyed good health for some years, and is therefore prevented from accepting many of the invitations which he receives to lecture abroad. The present award is a timely recognition of his great services to analytical chemistry, particularly as he will celebrate his seventieth anniversary next year.

Glass Technology at Sheffield: Mr. Michael Parkin

WITH the retirement of Mr. Michael Parkin, the Department of Glass Technology in the University of Sheffield lost its last member of that small team of pioneers recruited by Prof. W. E. S. Turner in the years immediately following the First World War to build up a department which has become world-famous. Mr. Parkin studied chemistry in the University of Sheffield; however, his studies were interrupted by war-time services in an explosives factory and in the Royal Flying Corps. In 1920 he joined the Department of Glass Technology, and apart from a short period in industry (he was works chemist to Messrs. Barr and Stroud, Ltd., 1924–28) he has served the Department continuously. Until 1955 the Department performed dual functions, carrying out the work of a University department and advisory work and investigations for the industry under the advice of the Glass Delegacy, the members of which were roughly equally divided between the University and the glass industry. Mr. Parkin made a major contribution to this work. This prevented his taking a direct personal responsibility for the research side of the work of the Department; but those whose responsibility it was to direct the research work would be the first to acknowledge the important part played by Mr. Parkin in assisting research workers. In 1955 the industrial work was taken over by the newly formed British Glass Industry Research Association, a step which Mr. Parkin never pretended to approve, but, as all who know him would expect, he has, during these past four years, spared no effort to help the University Department in its new regime to flourish. Perhaps the value which his present colleagues place on his services can best be emphasized by saying that he has been persuaded to continue as a part-time member of the staff for a short period while certain plans for future staffing of the Department mature.

British Association Representatives in the U.S.S.R.

THE British Association for the Advancement of Science has accepted an invitation from the U.S.S.R.–Great Britain Society, conveyed through the Soviet Embassy in London, to send two representatives to the U.S.S.R. to visit schools, universities and scientific institutions, to meet Russian scientists and to discuss future relationships and exchanges. The British Association's representatives are Dr. W. E. Swinton, who is an honorary general secretary of the Association, and Sir George Allen, who is its secretary.

European–American Nuclear Data Committee

A COMMITTEE for European–American Nuclear Data has been set up by the European Nuclear Energy Agency, in agreement with Euratom, the United States of America and Canada, to assure collaboration among members and associate countries of the Organization for European Economic Co-operation in the measurement of nuclear properties. The Committee will be primarily concerned with measurements of nuclear cross-sections and other basic data essential for the technical development of nuclear energy. The Committee is to consist of thirteen experts from the United States, Canada, the United Kingdom, the Euratom and other O.E.E.C. countries. Its operations will be in accordance with existing bilateral agreements. The work of the Committee will include the critical review of existing knowledge of nuclear cross-sections and constants, and of facilities, techniques and man-power available for their determination. The Committee will also collect and correlate data from available sources, seek to establish a standard nomenclature and methods of presentation for such data, and recommend and sponsor, as necessary, technical meetings and symposia to further its objectives. Finally, the Committee will promote the pooling and exchange, where appropriate, of equipment and personnel. Further information can be obtained from the Organization for European Economic Co-operation, Château de la Muette, 2 rue André-Pascal, Paris XVI^{e}.

New Forensic Science Society

A DECISION to form the Forensic Science Society was taken at a well-attended meeting held at the University of Nottingham on October 31. The object of the Society is to advance the study and application of forensic science in all its branches. With this aim in view, a series of symposia, to be held alternately in London and in the provinces, is being arranged.

Among the subjects suggested for discussion are blood, hypoglycæmia, street accidents and instrumentation. All persons professionally interested in forensic science are eligible for membership. The president of the Society is Dr. J. B. Firth, and the secretary Dr. E. G. C. Clarke, of the Royal Veterinary College, London, N.W.1, from whom further information can be obtained.

Preservation of the Malvern Hills

As a result of the confirmation, by the Minister of Housing and Local Government, Mr. Henry Brooke, of an order made by the National Parks Commission under the National Parks and Access to the Countryside Act, 1949, about forty square miles of the countryside in the counties of Gloucester, Hereford and Worcester, including the whole of the Malvern Hills, are to be established as an 'area of outstanding natural beauty'. The designated area extends from Knightwick in the north to Bromsberrow in the south and from Suckley, Cradley, Coddington, Wellington Heath and Ledbury in the west to Welland and Great Malvern in the east. It includes such well-known features as the Worcestershire Beacon, North Hill and the National Trust's property at Midsummer Hill. The responsibility for preserving the landscape rests with the County Councils of Gloucestershire, Herefordshire and Worcestershire as the local planning authorities. Government grants can be made at the rate of 75 per cent towards the cost of treating derelict land, tree planting and preservation and removing disfigurements. Grants are also available towards expenditure incurred in making agreements or orders for public access to open country and in appointing wardens. Designation does not provide any right of access to land not already open to the public. Nor does it affect the existing use of land, such as the use of War Department land for military purposes.

Newly Available Endocrine Preparations

The Endocrinology Study Section of the National Institutes of Health has the following highly purified pituitary hormones available for distribution free to qualified investigators: growth hormone, bovine, non-sterile for animal experiments only; follicle-stimulating hormone, ovine, sterile preparation: 25 mgm. vials for experiment, 5 mgm. vials for assay standard; luteinizing hormone, ovine, sterile preparation, 10 mgm. vials; prolactin, ovine, sterile preparation, 25 mgm. vials. Further information can be gained from Dr. R. T. Hill, Executive Secretary, Endocrinology Study Section, Division of Research Grants, National Institutes of Health, Bethesda 14, Maryland.

National Science Foundation: Grants for Private Foundations in 1957

Grants made by the National Science Foundation for scientific research and development by private philanthropic foundations and voluntary health agencies totalled 95 million dollars during 1957, of which about 59 million dollars was in support of basic research (No. 15, Reviews of Data on Research and Development. National Science Foundation, Washington, D.C.). It is estimated that research expenditure for 1957 by private foundations and health agencies in the United States amounted to about 8 per cent of the estimated national basic research expenditure of 700–800 million dollars. Expenditures for research and development by these institutions amounted to less than 1 per cent of the total expenditures for research and development by all organizations. Of 4,067 private foundations surveyed, 438 reported research and development programmes, and a total expenditure of 72 million dollars. Twelve foundations accounted for more than half this expenditure. 82 per cent was in the form of grants and related administrative expenses to outside organizations. The latter were predominantly educational institutions and their affiliated professional schools and hospitals. One in five foundations with research and development programmes reported expenditure for research in their own laboratories or facilities. The major part of support by foundations in 1957 covered the life sciences, accounting for 45 per cent of their total research and development expenditures. The social sciences were next in volume of support, and the physical sciences last, according to the report. Twenty-five of the thirty voluntary health agencies surveyed for 1957 reported expenditure for research and development; this amounted to 23 million dollars, of which almost one-half was for basic research. Four of the health agencies accounted for more than four-fifths of the total research expenditures, most of which were in the form of grants to outside organizations and individuals. Educational institutions and affiliated medical schools and hospitals were the major recipients. The voluntary health agencies concentrated almost exclusively on the support of biological and medical research.

Illuminating Engineering Society

At the meeting of the Illuminating Engineering Society held in London on October 13, Mr. H. G. Campbell was installed as president of the Society for 1959–60. Educated at Oundle and Queens' College, Cambridge, Mr. Campbell is managing director of Benjamin Electric, Ltd., and a director of Holophane, Ltd. The Leon Gaster memorial premium of the Illuminating Engineering Society for 1959 has been awarded to Dr. R. G. Hopkinson and Mr. J. Longmore (both of whom are with the Building Research Station of the Department of Scientific and Industrial Research) for their paper entitled "The Permanent Supplementary Artificial Lighting of Interiors".

U.S. Society of Protozoologists

The following officers, for the academic year 1959–60, were elected or appointed at the annual meeting of the Society at Pennsylvania State University, during August–September: *President*, Dr. Norman D. Levine (University of Illinois); *Vice-President*, Dr. Reginald D. Manwell (Syracuse University); *Executive Committee* (*new members*), Dr. E. R. Noble (Santa Barbara College, California), Dr. Charles Ray, jun. (Emory University).

Mond Nickel Fellowships

The Mond Nickel Fellowships Committee announced recently the award of a Fellowship for 1959 to: Mr. D. J. O. Mann (John Lysaght's Scunthorpe Works, Ltd.), to study the practical applications of recent metallurgical research and techniques to the production of basic semi-finished steel; and Mr. N. J. B. Pocock (Capper Pass and Son, Ltd.), to study developments in extractive metallurgy in the United Kingdom, Europe, the United States and Canada, and their dependence on the size and location of the organizations concerned.

University News: Hull

THE Department of Scientific and Industrial Research has made a grant of £6,000 to the Department of Chemistry towards the purchase of a mass spectrometer in support of research by Dr. G. C. Bond.

The Nature Conservancy has made a grant of £4,900 to the Department of Geography for a three-year investigation into the coastal geomorphology of Holderness and Spurn Head.

The following appointments to lectureships were made and took effect as from October 1: F. J. Bryant (physics) and I. C. Williams (zoology).

London

THE following titles are announced: that of professor of physics in the University of London, conferred on Dr. M. Blackman, in respect of his post at the Imperial College of Science and Technology; of professor of physical chemistry in the University of London, conferred on Dr. F. C. Tompkins, in respect of his post at the Imperial College of Science and Technology; of reader in biochemistry in the University of London, conferred on Mr. S. P. Datta, in respect of his post at University College; of reader in applied mathematics in the University of London, conferred on Dr. C. W. Kilmister, in respect of his post at King's College.

Oxford

RESEARCH grants are announced as follow: by the Medical Research Council, a grant not exceeding £1,100, for one year as from October 1, for scientific assistance in a study by X-ray analytical methods of insulin and related structures, to be carried out in the Laboratory of Chemical Crystallography under the direction of D. M. Hodgkin, reader in X-ray crystallography; by the United Kingdom Atomic Energy Authority, a grant not exceeding £5,700 for three years as from October 1, 1959, for studies in interferometric spectroscopy, to be carried out in the Clarendon Laboratory under the direction of H. G. Kuhn, also a further grant not exceeding £1,250 during the period October 1, 1959, to September 30, 1960, for work on the constitution of bismuth-rich alloys, being carried out in the Department of Metallurgy under the direction of Prof. W. Hume-Rothery; by the U.S. Public Health Service, a sum of 14,160 dollars for one year from September 1, 1959, for the continuation of research on vision and light quanta being carried out in the Department of Physiology by M. H. Pirenne, under the direction of Prof. E. G. T. Liddell.

The Department of Scientific and Industrial Research has provided grants not exceeding: £1,000 for one year as from October 1, 1959, for research on some natural products with biological activity, to be carried out in the Sir William Dunn School of Pathology under the direction of Dr. E. P. Abraham; £1,500 for one year as from October 1, 1959, for research into perceptual limitations in high-speed performance in the Institute of Experimental Psychology by H. Kay, under the direction of Prof. R. C. Oldfield; £22,860 for three years ending September 30, 1962, for an investigation of the geological age of rock series by methods based on natural radioactivity, being carried out in the Department of Geology under the direction of Prof. L. R. Wager; £1,220 for equipment for research on the biochemical mechanism of cell division, to be carried out in the Department of Biochemistry under the direction of Sir Hans Krebs, and £25,725 for the period October 1, 1959, to July 31, 1962, for an investigation of materials, using magnetic resonance and double resonance techniques, to be carried out in the Clarendon Laboratory under the direction of Prof. B. Bleaney.

The Admiralty has supplied, for the year ending March 31, 1960, a grant not exceeding £9,534 10*s*. 0*d*., for the continuation of research on centimetre waves and fundamental problems being carried out in the Clarendon Laboratory under the direction of Prof. B. Bleaney; and the Ministry of Supply a grant not exceeding £1,175 for a year from September 1, 1959, for the continuation of an investigation of fluorocarbohydrates being carried out in the Department of Biochemistry under the direction of Dr. P. W. Kent.

Announcements

MR. F. C. BRABY, chairman and managing director of Fredk. Braby and Co., Ltd., has been elected chairman of the Council of the British Non-Ferrous Metals Research Association in succession to Dr. Maurice Cook, who retires from that office on December 31.

THE Infra-Red Development Co., Ltd., Welwyn Garden City, has amalgamated with Hilger and Watts, Ltd., 98 St. Pancras Way, Camden Road, London, N.W.1. The Infra-Red Development Co. was founded in 1946 and specializes in the analysis of gases by non-dispersive infra-red techniques, primarily for industrial purposes; it has been under the technical control and management of Mr. W. B. Bartley, who will remain managing director. It will continue to operate at its works and offices in Welwyn Garden City.

THE well-known instrument makers, Griffin and George, Ltd., Ealing Road, Alperton, Wembley, Middlesex, are opening a new branch at 626 Welbeck Road, Walker, Newcastle upon Tyne. To celebrate this step, two exhibitions are being held, at the Heaton Assembly Rooms, Heaton Road, Newcastle upon Tyne, during November 17–20, and at the Corporation Hotel, Corporation Road, Middlesbrough-on-Tees, during November 23–25. The exhibitions will show a representative range of equipment of the latest type for education, research and development and industry.

UNDER the provisions of the Fulbright Programme, travel grants are available to citizens of the United Kingdom and dependent territories, to go to the United States for an academic or educational purpose, provided that they have adequate financial support in dollars for the visit, and have been accepted by an American institution of higher learning. Grants cover the cost of direct travel between the candidate's home and the American university or institution. They are available during June 1, 1960–August 15, 1960, for which applications must be submitted by March 14, 1960; and August 16, 1960–April 1, 1961, for which applications must be submitted by June 1, 1960. Application forms and further information can be obtained from the United States Educational Commission in the United Kingdom, 71 South Audley St., London, W.1.

WE regret that in the article entitled "Scientists in the Public Service in Britain" in *Nature* of September 19, p. 858, the statement issued officially relating to Dr. J. W. G. Lund is incorrect. Dr. Lund is in charge of the algological research of the Freshwater Biological Association, of which Mr. H. C. Gilson is director.

BOOTS' NEW BIOLOGICAL RESEARCH LABORATORIES

By Dr. G. I. HOBDAY
Director of Research

DURING a night-time air-raid on Nottingham in May 1941, nearly all the research facilities of Boots Pure Drug Co., Ltd., were destroyed. Blast and fire reduced virtually all the chemical and biological laboratories to heaps of charred rubble. The timing of this disaster could not have been more unfortunate. Under the stimulus of the national emergency we were expanding our research operations to enable the company to manufacture many vital drugs of European origin, supplies of which had been cut off by the War; penicillin, in the development of which we were to collaborate, was about to emerge. It was urgently necessary, therefore, to re-house our chemists and biologists as soon as possible. Because new construction was out of the question existing premises had to be converted. Choice of these was not easy since the Research Department was by no means the only one to suffer damage and every foot of space in the company's buildings in and around Nottingham was at a premium.

After due consideration, it was decided to re-house chemistry and biochemistry in a nearby building on the Island Street site and to evacuate the biological facilities, including bacteriology and pharmacology research and standardization, to a heterogeneous group of company buildings in West Bridgford, a Nottingham suburb. These latter, on which over the years a considerable amount of money has been expended, have served us well. Of course, it was evident from the beginning that they would answer our needs for only a limited period. But, as with many war-time expedients, building restrictions in the post-war years and the subsequent need to share out the capital cake among an increasing number of growing members of the company left us in occupation of them for longer than was originally intended. However, after about eighteen months of active planning we started construction of a new biological research building in September 1956, and occupation of it has just been completed.

The new building, which was designed by Boots' architectural staff, is sited in Nottingham a short distance away from the chemical manufacturing plant, but near enough so that services such as steam, electricity and water are drawn from the central works supply. It is near the present chemical and biochemical research laboratories and immediately adjacent to the site on which these facilities will be re-housed within the next few years. In plan, the building is in the form of an irregular H; one wing of four floors houses administration offices; Medical Department, library, canteen, conference room and a fully equipped lecture theatre seating 200; the linking block contains the main staircase and lifts together with lavatories and cloak-rooms; the other wing, longer than the first and on seven floors with a basement, is entirely laboratories. The total floor area is approximately 90,000 sq. ft. Externally, the building is of striking appearance; one wing is of brick and the other is faced with ceramic tiles in a checker-board pattern of grey and yellow. In internal design the administration wing and linking block are fairly conventional, but features of special interest include the pre-stressed concrete main staircase and the undulant ceiling in the canteen. The lecture theatre, which is acoustically designed, is fitted with stackable chairs which can be removed, entirely liberating the floor space for exhibitions, etc. Also to be noted is the pleasant medical and biological library housing 15,000 volumes, the stack room of which is fitted with mechanically operated stacking for economy of floor space. The décor throughout is modern, but not aggressively so, using mainly monochromatic treatments and avoiding disturbing colour contrasts. In the entrance lobby is a large colourful mural, abstract in design but intelligibly depicting the scientific disciplines provided for in the building.

The laboratory wing is functionally planned to accommodate the biological procedures employed, the common links throughout being laboratory animals and micro-organismal techniques. Required floor area and available site area comparison clearly pointed to a multi-storey building; this was quite acceptable, indeed in many respects desirable, since it permitted a plan of isolation of different functions on separate floors while reducing circulation distances

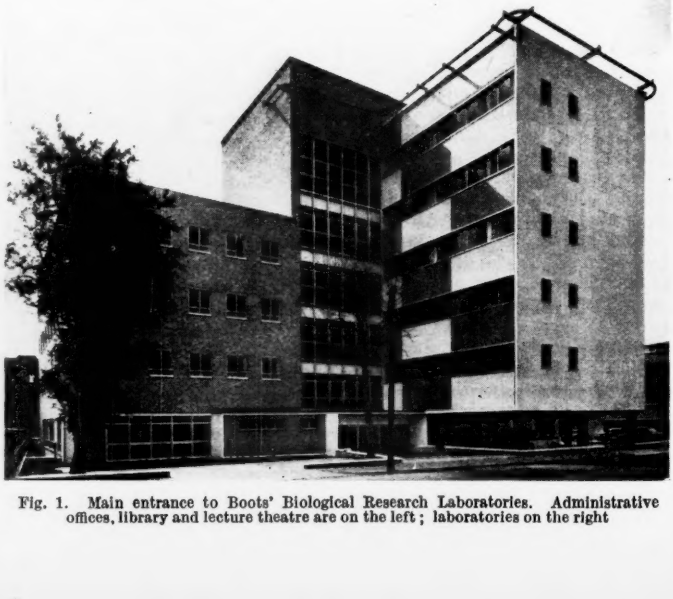

Fig. 1. Main entrance to Boots' Biological Research Laboratories. Administrative offices, library and lecture theatre are on the left; laboratories on the right

and simplifying centralized servicing. The floors were planned on a 9-ft. module in a block 200 ft. long and 45 ft. wide. This plan gave a spinal corridor arrangement which again helped in the separation of functions, since it was easy to form the areas at both ends into isolation units. A steel-framed building was chosen with space-frame girders 3 ft. 6 in. deep and 45 ft. span. By this arrangement none of the internal walls is part of the structure, permitting alteration of the working areas as the need arises; it also provides a space between ceiling and floor above in which services are distributed. Vertical distribution of services is through a duct, 10 ft. by 17 ft., which goes right through the building from top floor to basement. The whole of the laboratory wing is air-conditioned, inlet air being drawn in through an electrostatic precipitator and distributed through two separate systems, one serving the east side and the other the west side to give flexibility in taking care of solar gain through the large unopenable windows. A plenum system is used with pressure differences carefully arranged especially in laboratories where micro-organisms are used. All extracted air from areas of possible infection is filtered sterile before being vented to atmosphere. Where necessary, 'hospital' finishes on walls and ceiling are employed to facilitate cleaning and sterilizing; floor coverings are sheet polyvinylchloride in laboratories and hard asphalt in animal rooms and wash-down areas. Stainless steel benches and steel under-bench fittings are employed in all sterile areas and teak tops with wooden furniture elsewhere.

Equipment cleaning and sterilizing are centralized in the basement, where all refuse is incinerated. There are two systems of automatic hoists for handling separately dirty equipment to the basement and returning clean equipment to the requisite floors. All equipment and material passing from the isolation areas are heat-sterilized before proceeding down the 'dirty' hoist. Media-making is centralized and from a food store on the top floor animal diets are delivered to appropriate floors by chutes.

Facilities for work with radioactive labelled substances are provided in a self-contained suite. The main units of this are a synthetic chemical laboratory, a biological laboratory with separately vented cage cabinets and a radioactivity measurement laboratory. The main radioactive store is in a shielded room in the basement.

The work in the laboratories lies in the fields of pharmacology, toxicology, bacteriology, mycology, virology and parasitology. Some routine testing and standardization work is done on chemical and pharmaceutical production material, such as sterility-testing of injections and bio-assay of insulin. Otherwise the work is investigation, much of it comprising the biological component of research projects involving other research divisions. For example, in the field of parasitology trypanosomiasis is a major project, and chemical substances synthesized in the nearby chemical research laboratories, or new antibiotics isolated in the antibiotic research unit, are screened in the new building by specialized laboratory tests. Those of potential value will undergo more specific tests for activity and, in another unit in the building, for toxicity. Any worthy of clinical or field trial will be passed over either to the Medical Department or to the veterinary research division at Thurgarton, about ten miles outside the city. Work of this kind has produced 'Ethidium' and 'Prothidium' for treatment and prophylaxis of bovine trypanosomiasis. In a similar way the parasitologists working on amœbiasis have contributed to the development of 'Entamide' for the treatment of amœbiasis. Likewise the bacteriologists have collaborated with the chemists and pharmacists in developing a new antibacterial substance, 'Dybenal'.

Team operations of this kind provide much of the impetus for progress in the search for new substances for the treatment of human, veterinary and plant diseases. The new laboratories form a vital link in the chain of investigations between the first conception of a new drug and its final availability to the public. They serve the future in providing the type of working accommodation which the young research scientists of to-day expect and need in order to make their most effective contributions in the fight against disease.

THE BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION

THE British Food Manufacturing Industries Research Association Laboratories at Leatherhead were open to members on September 16 and to invited guests on the following day. The wide range of the research programme of the Association was demonstrated, the exhibits covering work in progress for the eight main groups into which the membership is divided, that is to say, cocoa and chocolate, sugar confectionery, meat and fish products, jams and jellies, pickles and sauces, oils and fats (including margarine and compound cooking fats), bakers' prepared materials and miscellaneous products ranging from table jellies to salted nuts and potato crisps. The work undertaken covers fundamental chemical, physical and bacteriological investigations alongside technological aspects of food manufacture.

For the chocolate industry an item of major importance is the study of the rheology of molten chocolate. An experimental viscometer was on show which had been designed and built to the requirements of the Association to give measurements of viscosity over a wide range of rates of shear. A method of plotting the viscometric data has been developed which leads to the flow properties of chocolate being expressible in terms of two constants. The glyceride composition of cocoa butter is being studied by chromatographic techniques. Complete separation of the mono-unsaturated triglycerides has been achieved by reverse phase paper chromatography using a non-polar stationary phase and a suitably chosen mobile phase. An investigation into the volatile constituents responsible for the flavour of cocoa and chocolate by gas chromatography is in its initial stage.

The properties of high-boiled sweets, particularly their behaviour on exposure to the atmosphere, is being investigated by means of an apparatus which

permits samples to be boiled under closely controlled conditions at low moisture content. The drying of certain types of confectionery deposited in starch moulds is being studied in two ways with the view of reducing the 'stoving time' necessary for their production. First, the effects of temperature and humidity on the rate of drying of gelatine and starch gums are being studied in a cabinet in which these factors can be closely controlled. Secondly, as corn-starch is used as a moulding medium, an investigation of its equilibrium relative humidity at elevated temperatures is of importance, and an apparatus for carrying this out has been devised and built by the staff of the Association. Other items of research connected with sugar confectionery manufacture are concerned with the properties of glucose used in confectionery (corn syrup), particularly the prevention of foaming on boiling and the tendency for the material to darken on storage.

The programme of research for the meat products group includes biochemical studies on residual tissue respiration and the measurement of oxidation-reduction potential. Colour changes and fading in cooked cured-meats are being studied with the aid of reflectance spectrophotometry. On the technological level, cooking properties of sausages and their colour stability during marketing are being investigated.

The Association's bacteriological laboratory is concerned on one hand with problems connected with bacterial spoilage of food, including the 'blowing' of canned goods, greening in cooked cured-meats and bacterial growth in vacuum-packaged bacon, and on the other hand, with bacterial aspects of the curing and processing of meat. Members are also advised on questions relating to general factory hygiene.

Problems connected with the canning of herrings are being studied by members of the Association's staff at the Torry Research Station, Aberdeen. The work involves chemical studies of herring flesh and the correlation of changes in the constituents of the flesh with flavour changes and with changes in texture which occur during the canning process.

The Association maintains close contact with horticultural stations concerned with the breeding and development of new varieties of soft fruits and helps the industry to assess their suitability for jam manufacture. The tendency for raspberry seeds to go 'blind', that is to say, become less visible, in jam is another problem which has engaged the close attention of the Association.

Much of the work in connexion with pickles and sauces is concerned with microbiological spoilage, and spoilage organisms from a large number of spoiled packs have been isolated and identified. Problems connected with the production of low-acid pickles involve studies of the pasteurization procedures necessary for a product which will combine adequate shelf-life with desirable appearance and eating properties.

In conjunction with the National Institute for Research in Dairying, an instrument for comparing the 'spreadability' of margarines has been developed. This instrument, the '*Fira/Nird*' extruder, has proved to have applications in connexion with other foods and, indeed, in other industries, where the rheological properties of semi-solid materials are important.

The Association maintains an extensive library of books and periodicals. Research reports and other publications are available only to members of the Association, who also receive monthly issues of abstracts from current scientific and technical literature. Some two thousand items are abstracted in a year.

B. R. Knapp

THE STRUCTURE AND CHEMISTRY OF PROTEINS

SYMPOSIUM ON PROTEINS AT PARKVILLE, AUSTRALIA

THE intensification in recent years of research relating to the utilization of the primary products of Australia, wool, meat, wheat and milk, and in medical research, resulted in the organization during September 10–11 of a symposium on "The Structure and Chemistry of Proteins", at the Division of Protein Chemistry of the Commonwealth Scientific and Industrial Research Organization Wool Research Laboratories, Parkville, Victoria.

The meeting was well attended, with eighty-five delegates participating. Several overseas visitors were present, including some who had attended a symposium on "Hæmatin Enzymes" in Canberra, immediately preceding the symposium on proteins.

The topics of the twenty-two papers presented ranged over many of the fields currently being investigated in other parts of the world, a notable exception, however, being studies of amino-acid sequences. This reflects the pre-occupation of Australian workers with the isolation and characterization of protein components from natural products as a necessary first step to a more comprehensive understanding of their structure. Although the complex protein mixtures of these products are the focal point of much of the Australian research, studies involving purified soluble proteins, such as insulin and lysozyme, plasma albumin and other proteins as well as synthetic peptides are, however, also in progress. The rapid advances which are being made in our knowledge of the amino-acid sequence, structure and behaviour of these classical proteins assist in the understanding and interpretation of the chemistry of the more complex biological systems.

The contributions that X-ray, infra-red and electron-microscope investigations have made in the study of the structural organization of keratin were illustrated by the work of R. D. B. Fraser, T. P. MacRae and G. E. Rogers. The application of X-ray crystallography to the study of the three-dimensional structure of a simpler compound, toluene-*p*-sulphonyl-L-prolyl-L-hydroxyproline monohydrate, was described by J. Fridrichsons and A. McL. Mathieson, and this contribution emphasized the stereo-chemical problem encountered with prolyl residues in a polypeptide chain. The properties of protein complexes of the insect cuticle were described by R. H. Hackman and interest was aroused in the nature of their strong bonding to chitin and quinones. The isolation

of nucleoproteins and labile plant viruses from leaves and their susceptibility to degradation by salt were discussed by J. W. Lyttleton.

The chemistry of thiols and disulphides is a prominent feature of protein chemistry and was exhaustively discussed. S. J. Leach and J. M. Swan described the important analytical advances made in this field with the aid of the polarograph and the preparative applications of sulphite in the presence of an oxidizing agent, such as cupric ions. The various methods of splitting disulphide bonds and their application in the extraction of soluble proteins from wool were discussed by J. M. Gillespie, I. J. O'Donnell and E. O. P. Thompson, and H. Lindley reported on the varying reactivity of the disulphide bonds of insulin. J. M. Creeth and D. J. Winzor were concerned with the specificity of the reaction of iodine with the sulphydryl groups of ovalbumin, while the important role of thiols in disulphide interchange reactions was clearly apparent in the experiments of F. J. R. Hird, R. Frater and J. R. Yates on the nature of cohesive forces in dough. Disulphide interchange was also responsible for the inhibition of several —SH enzymes by 'sulphanilamide disulphides' in an investigation reported by E. Boeri and L. Brighenti.

The physico-chemical characterization of proteins isolated from naturally occurring mixtures was covered in a further series of papers. The aggregation and disaggregation of soluble proteins and the changes induced during denaturation figured prominently in the discussions of papers presented by J. M. Creeth and L. W. Nicol on urease, by B. S. Harrap, I. J. O'Donnell and E. F. Woods on soluble wool proteins and by H. A. McKenzie on various enzymes and globular proteins. The various techniques used to follow conformation changes were critically examined and it became clear that the behaviour of a particular protein in any given system was not necessarily indicative of the behaviour of other proteins in the same system. The surface denaturation of proteins was discussed by F. MacRitchie and the application of the spread monolayer techniques to a comparison of the surface chemical properties of various cereal proteins was described by N. W. Tschoegl. The preparative applications of electrophoresis were illustrated by the work of J. F. O'Dea on the isolation of components of serum while P. R. Carnegie and R. L. M. Synge described the electrophoretic behaviour of cupric complexes of oligopeptides and a possible method for selectively isolating dipeptides from mixtures of peptides.

Chromatography as an aid in the purification of proteins was introduced by A. G. M. Marr, who described the fractionation of serum proteins on 'DEAE-cellulose'. D. H. Simmonds has applied this technique very successfully to the water-soluble flour proteins and he also reported amino-acid analyses of the various fractions using ion-exchange chromatography with an automatic recording apparatus capable of handling eight ion-exchange columns eluted simultaneously. The continued interest of chemists in the quantitative analysis of protein constituents was evident in this paper and that of J. H. Bradbury on an alternative method requiring paper chromatography of dinitrophenyl amino-acids. This method was particularly applicable to the estimation of amide groups.

G. Coleman and W. H. Elliot described their work on the synthesis of α-amylase by *Bacillus subtilis* and C. J. Shepherd discussed the effect of inhibitors of protein synthesis in *Aspergillus nidulans*.

A feature of the symposium was a lecture by Dr. R. L. M. Synge on "Naturally Occurring Peptides and Their Biological Significance". Although the search for naturally occurring peptides has not been intensive it was apparent from the stimulating survey by Dr. Synge that many unusual types of small peptide are already known. He went on to stress the necessity for quantitative data in the study of protein synthesis and expressed concern at the lack of experimental documentation for many of the generalizations by biochemists regarding the synthesis of proteins. A spirited discussion ensued and was continued in a subsequent session on protein synthesis.

Participants in the symposium were fortunate in having a range of papers presented covering most of the rapidly growing areas of investigation in the protein field and it may be hoped that similar conferences will be held in the future.

E. O. P. Thompson

ELECTRONICS EXHIBITION

The fourteenth Exhibition of Electronic Devices, organized by the Northern Division of the Institution of Electronics, was held at the Manchester College of Science and Technology during July 9–15. This annual exhibition is now well established and it provides an opportunity for new electronic apparatus to be demonstrated in rather less crowded conditions than obtain at the Physical Society Exhibition held in London during January.

A lecture programme was associated with the exhibition and, as is to be expected, a substantial part of this programme was devoted to transistor techniques. However, topics of general scientific interest were by no means excluded, and lectures on the argon chromatograph, on photographic densitometry and on the use of X-rays for micro-analysis were well attended.

The exhibition was divided into a manufacturers' section and a research section, the research exhibits forming much the smaller part of the whole. A relatively small number of research exhibits, especially from universities, has also been evident at the Physical Society Exhibition. Although research exhibits are of considerable general interest, and of special interest to those working in related fields, it is probable that for scientific workers as a whole the more important function of an exhibition of this type is to show instruments that are currently available. In the manufacturers' section this year's exhibition was notable for the extent of the exhibits of the various electronic agencies. These agencies handle the products of a number of manufacturers and they hold stocks of instruments and components. In the case of one agency the display occupied a whole room and included examples of the products of some sixty manufacturers.

Included in the new equipment on show were examples of 'second generation' oscilloscopes. Until

very recently, British manufacturers have been unable to offer high-performance oscilloscopes having trace brightness and amplifier band-width suitable for the display of single-pulses having fractional microsecond duration. This has meant that workers in such fields as nuclear physics and high-speed computing have either obtained equipment from North America or have constructed their own display systems. New types of oscilloscopes are now available from Messrs. Cossors, EMI, Marconi Instruments and Solartron. The cathode-ray tubes are mostly of the post-deflexion acceleration type and run at voltages of 6–10 kV., amplifier band-widths are 10–20 Mc./s., and the deflexion sensitivity at full gain is about 100 mV. per cm. This specification is adequate for all but the fastest applications, and for these, two manufacturers are offering oscilloscopes with distributed amplifiers having a band-width from d.c. to 40 Mc./s. Messrs. Cossor and Heathkit showed kits of parts that can be assembled to make items of test gear such as valve-voltmeters and simple oscilloscopes. The kits normally employ printed circuits, which simplify the wiring, and can be assembled with semi-skilled labour. A wide variety of silicon devices are now available, and Messrs. Ferranti showed a range of silicon photo-voltaic cells; these have a response time in the microsecond region and have applications in equipments using modulated light. The new cells have a high conversion efficiency and in the larger sizes can be used as solar cells to provide electrical energy from sunlight.

This annual exhibition continues to be well attended and it provides an opportunity for scientific workers in the north-west of Britain to keep abreast of current electronic equipment and components.

V. H. Attree

UNITED KINGDOM CIVIL SERVICE COMMISSION

THE ninety-third annual report of the Civil Service Commissioners, covering the year April 1, 1958–March 31, 1959, records an increase in the number of candidates successful in open competition from 13,057 to 14,616, but for the administrative class the number of successful candidates decreased from 39 to 37, though well above the 1956–57 figures, and some departments were short of recruits, although the number of unfilled vacancies is not large (Report of Her Majesty's Civil Service Commissioners for the period 1st April, 1958 to 31st March, 1959. Pp. 36. (London: H.M. Stationery Office, 1959.) 2*s.* 6*d.* net).

The Commissioners are continuing their efforts to attract a larger number of good candidates from the universities. Candidates in the limited competition for the administrative class further decreased in number. Recruitment to the senior branch of the foreign service was also disappointing, and the shortage of candidates for the statistician class persists. Less than 50 per cent of the declared vacancies as patent examiner have been filled and there was again a shortage of good candidates for scientific officer, engineering and draughtsmen posts, and many vacancies remain unfilled, particularly through a dearth of physicists. Grave shortages remain in the telecommunications and other electronic fields; however, there was a small increase in the number of candidates in the senior scientific officer competition and most of the vacancies which had been notified were filled.

Applications in the assistant experimental officer/experimental officer competition remained remarkably steady and generally sufficient candidates were successful to meet departmental needs. The supply of biologists again exceeded the limited demand. The research fellowship competition continued to attract interest from workers in all fields of research, and thirteen candidates were offered the award. There is some evidence that it is becoming harder to attract good applicants for junior fellowships.

Results of interviews in Ottawa and Washington in April 1958 to select applications for research fellowships and scientific officer posts were less satisfactory than originally appeared likely, and in the event only one candidate joined the Service as Research Fellow and one as a senior scientific officer, although some well-qualified men appear to have been stimulated to return to Great Britain in the universities or in industry.

EUROPEAN NUCLEAR ENERGY RESEARCH

THE seventh annual report of the Netherlands'–Norwegian Joint Establishment for Nuclear Energy Research*, describing the work of the Establishment during the period July 1, 1957–June 30, 1958, mentions that the research reactor, *Jeep*, was in almost continuous operation at 450 kW. during the year, with a total release of heat of 105·6 MW. days, but that the corrosive effects of the heavy water have grown worse and a minor leakage of heavy water occurred during April. The completion of the Halden boiling water reactor, which is an Institutt for Atomenergi project and which is situated inside a rock excavation near the paper pulp factory, Saugbrugsforeningen, will be delayed by about a year because of construction and design problems. The reactor tank was completed during the spring of 1958. The necessary amount of heavy water which was purchased from the United States of America is now stored at Halden, and part of the uranium ordered from Great Britain has been delivered. An agreement between Norway, Denmark, Sweden, Austria, Great Britain, Switzerland and Euratom, on the joint operation of the reactor, was signed by representatives on June 11, 1958.

Because of the higher demand for radioisotopes, and technical improvements in the production system, the number of isotope deliveries from Kjeller to customers outside the Establishment increased by 33 per cent over the previous year. The deliveries

* Seventh Annual Report, July 1957–June 1958, of the Netherlands'-Norwegian Joint Establishment for Nuclear Energy Research. Pp. 32. (Kjeller near Lillestrøm: Netherlands'-Norwegian Joint Establishment for Nuclear Energy Research, 1959.)

were mainly to the Scandinavian countries. Detailed information about the type of isotopes produced and their distribution is given in the report. Separate sections deal with the activities of the Chemistry, Metallurgy, Reactor Engineering, Physics and Health Physics Divisions. The chemical analysis of uranium and D_2O is now carried out on a routine basis and the spectrographical methods used for impurity control of medical isotope products and the determination of plutonium have been improved. The main task of the metallurgical group has been the production of UO_2 pellets, and in addition to considerable computational work and experimental tests connected with the Halden boiling water reactor project, the Physics Division has obtained new neutron diffraction data on U_3O_8 and U_4O_9. The Health Physics Division is responsible *inter alia* for the daily radiation monitoring in the laboratories; the radiochemical analysis of biological specimens; and the general medical check-up of personnel. Of the 191 persons controlled by the Division during the year, only one received a radiation dose exceeding 5 rems.

The Netherlands–Norwegian Reactor School was officially opened on April 12, and the first nine weeks standard course commenced on April 14 with twenty-eight students: fifteen from Holland, eleven from Norway and two from Switzerland. The construction of the new isotope building was started in January, and of an office building to house the ship propulsion group and the Engineering Division in April. As in former years, the Establishment benefited from the exchange of scientists with similar institutions in other countries. Seven guest scientists worked at the Joint Establishment for Nuclear Energy Research for the whole or part of the period under review. The two sponsoring organizations of the Establishment, the Reactor Centrum Nederland and the Institutt for Atomenergi, took part in international co-operation in the field of atomic energy, in particular, in the European Atomic Energy Society, the Organization for European Economic Co-operation, and the International Atomic Energy Agency. Summaries of the main activities of the two organizations are given in the appendixes to the annual report.

THE FRANKLIN INSTITUTE

THE Board of Managers of the Franklin Institute in presenting their annual report for 1958 (*Journal of the Franklin Institute*, **267**, 317; April 1959) express their gratification at the progress during the year in all the Institute's programmes of service to science, but point out that without increased funds the Institute cannot expand and may not be able to maintain its present activities. In addition to the Franklin Institute Laboratories and the Computing Center, both located in Philadelphia, the Institute owns and operates the Bartol Research Foundation in Swathmore, Pennsylvania, and is trustee for the Biochemical Research Foundation in Newark, Delaware. The Institute conducts basic and applied research on a contract basis for government, industry and private concerns, and the Computing Center co-operates with the Laboratories which carry out projects under contract in the fields of engineering and the physical sciences. The Bartol Foundation is concerned with the study of fundamentals in physics, low-energy exploration and cosmic phenomena, and the Biochemical Foundation with cancer research.

It was to be expected that public interest in the activities of the International Geophysical Year, and in rockets, satellites and space travel, would largely colour the work of the Education and other divisions of the Institute during 1958. Of the twenty-one lectures presented at the Institute meetings during the year, eight were on subjects related to the space age; the exhibit, "Progress of Time", contributed to the Institute's Science Museum by the Hamilton Watch Co., included the Mars Space Clock, and other loan exhibits showed the successful launching of the *Explorer* rocket, the *Vanguard* satellite, and the *Pioneer* lunar probe; new presentations in the Planetarium were "The American Satellite" and "Astronomy in History", and the staff of the Planetarium were responsible for the operation of the Institute's Moonwatch Station; and the series of ten semi-technical lectures on astronautics sponsored by the Astronomy Department were published in December as Monograph No. 6 entitled "Ten Steps into Space".

The Library of the Institute, which began as a small collection in 1824, now comprises 162,054 volumes, of which 3,054 were acquired during 1958—986 by purchase, 544 by gift and 1,524 by binding. New accessions included eighteen Russian periodicals, four in translated English editions, and a remarkable set of the owners' file copies of records and correspondence of the Penang Sugar Estates Co. (British Malaya), comprising twenty-three handwritten volumes of the work of the Company during 1876–97. A symposium on "Thermoelectric Effects" was held on September 8, primarily for research workers in this specialized field, and an all-day symposium on "Odour" on October 21 during the "Cleaner Air Week" in Philadelphia. Three new titles were added to the series of monographs published under the auspices of the *Journal*: "Particulate Emission", "The Airways Modernization Board—Its Mission and Methods" and "Ten Steps into Space" mentioned above.

Details are given in the illustrated report of the long-range research programmes and new developments of the separate research organizations. These include air-pollution research; thermoelectricity and semiconductors; electron microscope studies of dislocations in metals and zone refining of reactive metals; the physics of polymers; and flow loops in nuclear engineering. A novel etching technique has been developed for studying dislocation loops generated in metals under stress and their motion and growth in zinc have been filmed (*Journal of the Franklin Institute*, **267**, 335; 1959). A simple sonic-vibration method for the early detection of glaucoma has been demonstrated and an all-transistorized sensitive cane for use by the blind has been successfully tested. The report concludes with brief details of the finances of the Institute, the membership and staff.

ECONOMIC DEVELOPMENTS IN THE MIDDLE EAST

In a report dealing with developments for the year 1957–58, published by the United Nations as a supplement to the world economic survey of 1958, there is included a useful survey of agricultural production and development, industry, petroleum production, and foreign trade in the several main countries included in this politically highly sensitive world region (Iran, Iraq, Israel, Jordan, Lebanon, Saudi Arabia, Sudan and Turkey). About one half of the text deals with factual summaries, and about one half gives statistical tables (Economic Developments in the Middle East, 1957–1958. (Supplement to World Economic Survey, 1958.) Pp. viii+104. (New York: United Nations; London: H.M. Stationery Office, 1959.) 1.25 dollars; 9*s.*; 5 Swiss francs).

The information given regarding the key product, oil, has special interest. The rapid recovery and expansion of this industry during 1957 (after the decline in production as a result of the Suez crisis) is remarkable. In 1957 there was an increase over 1956 of only 3·7 per cent, but this advanced by a further increase of 20·7 per cent in 1958. The total production of the Middle East as a share in world production rose to 23·6 per cent in 1958 as compared with 20 per cent in 1957. The main contributors to the large rise in oil production were Iran and Kuwait, but though producing relatively small quantities, other countries have been expanding their output at a high rate, and a new entrant was Syria where, in the north-east of the country, a field was discovered in 1958 estimated to have an output capacity of about 2 million tons. Agricultural production in the same year shows continued expansion at the rate of about 3 per cent per annum, both in food production and in the output of industrial cash crops on which the several countries depend as a major source of foreign exchange. It is significant that the rate of growth in both exceeded the growth of population. A continued shift in the pattern of production towards industrial cash crops and greater use of fertilizers and agricultural machinery helped to maintain the rate of agricultural output, but the uncertainty of climatic conditions caused wide fluctuations.

Apart from the physical difficulties affecting production within the region, two comprehensive programmes of agrarian reform were started in the Syrian region and in Iraq late in 1958, and the social change and redistribution of income that these are likely to produce will very probably have a strong impact on the shape of future economic development. From these economic changes within, there emerges a picture of foreign trade and payments showing much variation, and with interesting evidence of change in operation leading to shifts in the geographical direction of trade, particularly of the cotton exporting countries; thus the United Arab Republic and Sudan moved away from Western Europe and the United States toward the U.S.S.R., Eastern Europe and the Far East. Iran, Jordan, Turkey and Israel, on the other hand, maintained their high share of trade with the United States and Western Europe, although an increasing proportion of the exports of Israel and Turkey went to eastern European countries in 1957 and 1958. Oil exports show a significant increase in their share to Asia and the Far East, though the dominant traditional markets in Western Europe are maintained. Alice Garnett

THE NATURE OF POLISHED METAL SURFACES

Hooke, Newton and Herschel all held that the asperities in a roughly ground surface are cut away during polishing, leaving a series of fine grooves; the finer the polish the finer these grooves or 'scratches'. Rayleigh agreed that the asperities are worn down but thought that the material is removed in an almost molecular fashion. In 1921 Beilby advanced the radical view that, instead of the asperities being worn away, the depressions in the surface are filled in by material which is smeared across the surface, covering it with a layer which he thought was glass-like or amorphous in character. This has come to be known as the 'Beilby layer'. The idea of the layer being truly amorphous has been modified slightly in more recent times, but the basic concept of a layer which is physically distinct from the substrate and which has lost its obvious crystalline properties is still retained.

Beilby did not propose any specific smearing mechanism, although he inferred that surface tension forces were responsible. A most plausible mechanism was afterwards advanced by Bowden and Hughes which was based on observations that very high local temperatures can be attained when two solids rub past one another. They suggested that asperities in the surface are melted when abrasive particles rub across them, the liquid so formed depositing in and filling adjoining depressions. It was further proposed that, due to very rapid chilling, this molten material solidifies in an amorphous-like condition.

A paper by L. E. Samuels reviews work carried out at the New South Wales Branch of Defence Standards Laboratories which strongly supports the earlier view that polishing is essentially a fine cutting process, and is believed to establish with reasonable certainty that the Beilby layer does not exist.

The new theory is that metallographic polishing occurs primarily by cutting, the individual abrasive particles acting in a similar manner to a planing tool. Material is removed and scratches are produced; the better the polish the finer the scratches. The surface is crystalline but deformed, the magnitude of the deformation decreasing with increasing fineness of polish to a surprisingly low level in the case of the finest polishes. Moreover, the deformation decreases rapidly with depth so that comparatively perfect material is exposed by a very light etch (*Austral. J. Sci.*, **21**, 6; 1959).

EEL MIGRATION

IT seems probable that many of the difficulties that have undermined Dr. Tucker's belief[1] in the ability of European eels to return to the Sargasso Sea would have disappeared if he had compared the European eel with the Atlantic salmon, the return of which to spawn after a migration of comparable difficulty is more readily demonstrable.

Salmon, both Atlantic and Pacific, migrate to feed in the sea: here they may stay for one and a half to four or more years. This active feeding period is followed by a spawning migration during most of which the animal does not feed. Fasting begins as the salmon nears fresh-water; the subsequent migrations upstream, often carried out under difficult physical conditions, are very fatiguing and call for a considerable expenditure of energy. Yet, after a migratory fast lasting up to a year, millions of salmon survive to partake in most energetic spawning activities. In fact, about 5 per cent return to spawn again, and a small proportion may spawn three times.

Eels spend most of their lives feeding in fresh-water. This feeding period of from six to twenty-five years duration is as clearly preliminary to spawning migration as are the years spent by the salmon in the sea. I do not agree with Dr. Tucker that European silver eels are starving and debilitated: the many thousands that I have handled have been vigorous, extremely energetic and in good condition. It is of interest that eel-dealers store living eels for long periods, yet these eels are in the end still fat enough to be sold as highly nutritious food.

On their 3,500-mile spawning journey, eels have to contend only with slow-moving ocean currents, not to be compared with the fast-flowing streams encountered by salmon. If eels travel at a modest 40 miles a day, the journey need take only thirteen weeks—not a long fast compared with that of many salmon.

Dr. Tucker claims that American eels, because of their larger size and apparently juvenile sex condition, are better suited than European eels for their spawning migration. But size is not a criterion of condition; salmon, ranging from 3½ lb. to more than 60 lb., complete their spawning migration successfully; many reach the rivers of Britain in an advanced stage of sexual maturity, fast, and survive to spawn. Recent examinations of the state of the gonads in large samples of eels have convinced me that the European silver eel is not "already well advanced towards being a reproductive oceanic fish". The gonads of silver eels are not in an advanced stage of development. Many silver eels migrate when their gonads are scarcely more advanced than those of yellow eels. In fact, the gonads of the silver eels are in about the same developmental stage as those of female salmon smolts and unspawned male smolts.

One of the most strongly emphasized points in Dr. Tucker's argument is that European silver eels are rarely caught at sea. But neither apparently are American eels. Nor is it surprising that eels at sea are elusive. They do not feed and so cannot be caught on long-lines, nor are they likely to stay captive in any normal deep-sea trawl. It is no cause for astonishment that eels are not caught in the Straits of Gibraltar, for no commercial fishing gear in use there can be expected to catch eels. Salmon are rarely caught off shore, and salmon should be much more catchable, for they feed in the sea, they stay there much longer and they are not so shaped as to make escape from nets easy. Yet countless millions reach their spawning grounds yearly, though the number caught in the open sea is very small.

Finally, Dr. Tucker's hypothesis requires that a large proportion of the American eel population is 'lost' yearly as a reproductive potential, since American eels which spawn in the wrong place produce progeny which become European eels and never succeed in spawning. If this were a true account, there would be intense selection in favour of eels which found the 'right' spawning ground. It would be very surprising if natural selection on this large scale had failed to eliminate the European eel in a few generations.

J. W. JONES

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[1] Tucker, D. W., *Nature*, **183**, 495 (1959).

I MUST emphasize at the outset that Dr. Jones's communication, even if it were acceptable in its entirety, contains nothing relevant to the fundamental problem of eel navigation and nothing which has any bearing upon my hypothesis that the two Atlantic *Anguilla* phenotypes may be environmentally differentiated and distributed without genetic intervention. For the rest, the difficulties which led to a heavily documented paper[1] are not likely to be dispelled by criticism which ignores not only literature already cited, some of it on two occasions[1,2], but also that relevant to its own substantiation. Moreover, a recent independent review[3] has made such a comparison as Dr. Jones demands, and without detriment to the new theory of eel migration.

Both the salmon and the eel undergo migrations which raise problems of navigation, physical effort, condition and osmo-regulation. Thereafter the comparison breaks down to such an extent that knowledge of one casts little light on the ways of the other. The eel is catadromous; the salmon anadromous. The first migration of the eel is as a larva passively transported in the surface layers; its second as a starving adult travelling in the deeper layers and probably by a different return route. Both of the migrations of the salmon are accomplished as an adult fish, travelling in substantially the same water-masses along the same routes and actively feeding until the final return to fresh-water. Eels are in peak condition shortly before the commencement of their final journey; salmon shortly before the end of it.

The European eel is, in Dr. Jones's view, an ocean traveller, accomplishing a long journey of at least 3,500 miles for the south-west European stocks and at most 5,000–6,000 miles for the White and Black Sea stocks. The longest recorded journey for an Atlantic salmon is 1,730 miles in 328 days[4]; characteristically, its migrations are much shorter—a few hundred miles along the coast or to and from feeding-grounds off the shelf—and fairly easily explained by internal changes in the osmo-regulatory mechanism

which prompt it to seek salt or fresh water and by a proved propensity to wander along the coast until it smells the outfall of its native stream. However arduous the last stage of the journey of the salmon up-river, it can and does alternate activity and rest and make use of slack water and pools for the latter purpose; for the eel in an opposing ocean current no such respite is possible without losing ground.

Segregation of breeding stocks of salmon provides excellent opportunities for adaptive variation and variation of inherited behaviour-patterns through genetic isolation; in the eel nothing of the kind is possible.

Given this summary of the habits of the two fishes, which further have very different patterns of locomotion and are widely unrelated, I see very little ground for generalization from one to the other; the analogy is rejected with good reason, but Dr. Jones should not assume that it was ignored.

Salmon survival for subsequent spawnings is due in part to the fact that degenerative changes in the gut are confined to the mucosa, which is renewed in kelts that recover[5]; in the eel the changes are profound and lead ultimately to complete destruction of the gut[2,6]. The personal findings of Dr. Jones, like those of Prof. D'Ancona previously dealt with[2], do not affect the evidence that the gut of the European eel is self-destroyed before the fish is more than a few hundred miles offshore.

Here a new point may be introduced. Experimental work on the eel has shown that in the sea it contrives to maintain effectively the internal environment of the freshwater fish. It does this by swallowing sea water, absorbing water and salts through the intestine, excreting an isotonic urine and discharging surplus chlorides through the secretory cells on the gills[7]. In European eels, once degeneration of the gut has proceeded merely far enough to impair the absorptive function, this mechanism can no longer operate; osmo-regulation must then depend wholly upon the relative impermeability of the body mucus and upon compensating liberation of water through breakdown of stored lipo-proteins. Failure of the whole apparatus would lead to rapid exsiccation and death. Experimentally induced failure, by preventing eels from swallowing sea water, does in fact produce an 11–14 per cent loss in weight and death within 3–4 days[7]. This situation, even more than the likely inadequacy of the food reserves to provide for locomotion, physiological work (for example, in chloride secretion) and gonad maturation, could account for the failure even of Mediterranean eels to reach apparently suitable breeding-grounds in that sea (*post*).

I am not subdued by Dr. Jones's experience with thousands of eels. The great curse of the voluminous work upon eels, upon the Salmonidae and upon sundry other animals, has been that too much of it has been mechanical and repetitive, replete with experiments unintelligently planned and mountains of data inadequately pondered, parochial alike in the range of its geographical experience and in its isolation from relevant literature from alien countries and related disciplines.

The physiology of the eel is not "clearly preliminary to spawning migration"; it could be, and I think is, atavistic and doomed to fruitless failure. We have no right to assume climax as normality in any incomplete behaviour-pattern or physiological process when climax has never been observed and no unequivocal circumstantial evidence of climax is available.

I agree that a 'stored' silver eel may well retain its condition like any other relatively quiescent animal; migrating eels, however, lose up to 20 per cent of their weight before they leave the Baltic[8] (reserve fat amounts to about 25 per cent) and this over periods greatly in excess of that shown to be necessary for the initial osmotic adjustment[7]. Either the food reserves are being rapidly used up, or there is a loss of water showing that the osmo-regulatory mechanism is already breaking down under the quite low salinities of the Baltic.

Dr. Jones suggests a hypothetical "modest 40 miles a day" over thirteen weeks for the eel migration. Norwegian work[9] summarized by Menzies[10] has shown that, of 598 long-distance journeys by marked and recovered salmon, 569 were accomplished at 5–25 miles a day and only twenty-nine at higher speeds up to 62 miles a day. Speeds for Baltic eels marked on a comparable scale[8] (the work was cited previously[1]) are in general between 5 and 10 miles a day; the record is 32·5 miles a day sustained over a mere two days.

Comparative data for condition of American and European eels are hard to come by. Vladykov[11] gives 411 gm. for the mean weight of Quebec bronze eels of 61 cm., compared with Frost's 425 and 414 gm. for 61 cm.[12] Windermere yellow and silver eels, respectively. The superficial agreement does not allow for the fact that the American eels cited are at an earlier and probably much younger stage, nor deny that the average weight of migrating American females is four times greater than that of the European, that their maximum sizes and weights are greater and their potential journey much shorter. I do not think we can avoid the conclusion that the American eels are better prepared. Dr. Jones's citation of the range of weights of salmon grilse is meaningless without an indication of the success-rates at different sizes and of the conditions overcome. Such data as are available for the migration speeds of the outward-bound salmon smolt[13] show that these are much slower than those of the larger returning grilse. Further, maximum velocity in fishes is a function of length and frequency of tail-beat; though the latter does fall with increasing size, larger individuals are faster swimmers[14].

My paper[1] mentioned merely "perceptible enlargement of the gonads", a statement in agreement with Dr. Jones's findings. It then proceeded clearly to specify those characters of migration livery and bathypelagic adaptation in which, by comparison with the American eel, the European may be regarded as advanced.

A surprising variety of fishes, including other Apodes, have been taken in deep-sea trawls by scientific expeditions; I have before me 29 *Synaphobranchus* from a single haul of an Agassiz trawl at 1,300 metres. There are at least 108 cases of records, with Scottish interest alone, of salmon taken at sea, 82 by trawls and various nets[15]. Salmon are surely less numerous than eels; being capable of a maximum velocity of 10 m.p.h. for short periods[16], compared with the eel's peak 2·6 m.p.h.[17], they should have greater chances of avoiding nets and not less. The infrequency of capture of eels in European seas remains significant. Non-capture of American eels is agreed; there is, however, no convergence of essential migration routes and intensive trawling comparable to that of north-west Europe. I did not write of "commercial fishing gear" in the Strait of Gibraltar but of an "intensive study"; still more explicitly,

scientific investigations by Danish, French and Monagasque expeditions.

Evolution by natural selection of a population which chose the 'right' spawning-ground would be conceivable enough in the case of, say, salmon in polluted and unpolluted tributaries of the Welsh Dee. The case of the eel is not so simple. The Atlantic *Anguilla* forms are believed to be environmentally differentiated by differences in the temperature stratification[1] and are certainly differently distributed by the various movements of the surface layers of the Sargasso between lat. 20° and 30° N. In the underlying deep waters in which the eels breed, the temperature and salinity conditions at a given depth are relatively uniform over a wide area; it is therefore unlikely that any sensory discrimination could pick a 'right' spawning-ground directly related to a 'right' surfacing-area. Selection in relation to travelling and ripening times could not have any genetic effect owing to the failure of the current-system to return the larvæ precisely to the parental starting points along the American coast. While the new hypothesis may seem more spectacular, it is statistically no more remarkable than the normally high infantile mortality-rate accepted in marine animals; the survival-rate is still sufficient to maintain the population. Parallel cases of expatriated populations failing to breed are the British *Octopus vulgaris*[18], the Norwegian *Palinurus elephas*[19] and the Lagos *Branchiostoma nigeriense*[20], all of which are maintained by immigrations of larvæ bred elsewhere and so represent similar cases of wasted reproductive potential.

There is likewise no ground for belief in selection producing genetic restriction of the eco-phenotypic variability potential of the Atlantic *Anguilla*, that is, eliminating the phenotypes with 110–119 vertebræ as such, as opposed merely to those going to Europe.

Some further evidence in favour of the new hypothesis is now briefly noted.

Possible parallel cases. Bruun[21] has commented on the hitherto unexplained coincidence that, of four pairs of Atlantic apodal 'species', having distributions roughly similar to those of the two types of *Anguilla* larvæ and, moreover, larvæ which can be taken "at the same place and same depth within a certain area of the Sargasso Sea", the American 'species' of each pair has the lower number of vertebræ (Table 1). This situation may well be due to a common eco-phenotypy rather than to coincident genetic effects.

Table 1. Numbers of Vertebræ or Myomeres in some North Atlantic Eels, mainly after Bruun (ref. 21)

	East Atlantic	West Atlantic
Anguilla anguilla	110–119	—
Anguilla rostrata	—	103–111
Conger conger	154–163	—
Conger oceanicus	—	140–149
Synaphobranchus kaupi	143–154	—
Synaphobranchus infernalis	—	131–140
Leptocephalus euryurus	111–119	—
Leptocephalus similis	—	104–113

Likewise, Tambs-Lyche[22], supporting the new hypothesis, has suggested that there is a similar pseudo-speciation in the Atlantic Paralepid fishes (order Iniomi).

Extended pelagic phases. 'European' eel larvæ grow to a greater length and unmetamorphosed age than the 'American' and are, on the new hypothesis, regarded as an extended pelagic phase of one species. Parallel cases of facultative prolongation of larval or juvenile life in marine animals under conditions unfavourable to metamorphosis occur in the surgeonfish *Acanthurus hepatus*[23]; the frogfishes *Antennarius* spp.[24]; the *Macrotritopus* larvæ of the benthic octopus *Scaeurgus unicirrhus*[25]; and in numerous Decapod Crustacea[26].

Selection of breeding-grounds by Anguilla *and* Conger. On the old hypothesis, *Anguilla anguilla* and *A. rostrata* were separate species with distinct breeding-grounds in the Sargasso Sea. The American *A. rostrata* and *Conger oceanicus* shared a common breeding-ground. The European *C. conger* shared the only breeding-ground of *A. anguilla*, but also used other breeding-grounds between Gibraltar and the Azores and inside the Mediterranean[27].

I have already noted the fact that *A. anguilla* does not breed in apparently suitable areas much nearer Europe than the Sargasso[1]. The paradox raised on hydrological considerations is now reinforced by *Conger conger* acting as a biological indicator. We may conclude that the 'European' eel is not breeding in the Sargasso, any more than it is in the Mediterranean or Eastern Atlantic.

Location of the Anguilla rostrata *breeding-ground.* I previously suggested that the *A. rostrata* breeding-ground had been placed too far west[1]. In support of this contention, be it noted that, until 1920, catches of *A. rostrata* larvæ from all sources totalled only 34 specimens[28]. In 1920 the *Dana* took a further 1,000 specimens; because, however, her track out of Porto Rico towards New York[29] followed the north-east boundary of the 15 mm. *A. rostrata* contour, it follows that data for and within that contour remain thoroughly inadequate. The probable breeding-ground between *c.* lat. 20–22° N., long. 50–60° W. has not been investigated at the proper season. Material of the other American Apodes cited above is likewise scanty by comparison with the European[21,27].

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MECHANISM OF ANIMAL JOINTS

Sponge-hydrostatic and Weeping Bearings

ANIMAL joints are very efficient pivots. Charnley[1] gives 0·01–0·02 as the friction coefficient of a human ankle joint and shows that this low friction does not result from hydrodynamic lubrication. He points out that ordinary boundary (thin-film) lubrication would produce five or ten times the observed friction and suggests that Nature may have discovered, in its combination of cartilage and synovial fluid, a system which is very slippery even under conditions of boundary lubrication. The purpose of this communication is to propose an alternative system, which is, in fact, an example of a new and interesting class of bearings. The principle is illustrated by the experiments to be described.

A piece of closed-cell sponge rubber (cell size 0·7 mm. and less) had one of its impervious cover sheets cut off to expose the cells. This surface, lubricated with soapy water, was placed against a glass plate with a loading of about 40 lb./in.². The friction coefficient was then measured as a function of the time for which the load had been applied (Fig. 1, curve *a*). The friction coefficient is extremely low, but rises in the course of an hour or so. If the sponge is separated from the glass for a few moments and then replaced, the friction falls to its initial low value. This is consistent with the idea that most of the load is supported in a frictionless manner by the little volumes of liquid trapped in the pores of the sponge. It is, in fact, a 'hydrostatic' bearing. The sponge material itself, being surrounded by liquid, is pressed against the glass only by its own stiffness. This is small enough to produce very little friction but still large enough to seal the cells (hydrostatic lubrication is not new, but previous arrangements have depended upon mechanical design to contain the lubricant and upon external pumps to pressurize it). The liquid pressure was measured and found to be about that required to support the load. Observation, through the glass, of the working face of the sponge clearly showed the water-filled pores. The movements of a particular volume of water could be traced by using dye. Substantial outward seepage of the water had occurred in an hour, which seems to explain the observed increase in friction with time. For purposes of comparison a sample of sponge rubber with open cells, but otherwise of about the same texture, was used. This should allow much greater seepage of fluid. Note (Fig. 1, curve *b*) the tremendous increase in the rise of friction. Lastly, to check the technique of measuring friction, a piece of closed cell sponge was tested with its cover sheets intact (Fig. 1, curve *c*). This shows the high value expected for plain rubber once the wring-out of lubricant established boundary lubrication conditions.

A difficulty arises if one attempts to explain animal joint lubrication by this principle. The animal joint appears to involve two similar surfaces rather than a hard, impermeable surface running against the equivalent of a sponge. Two sponges do not run very well against each other. Suppose, however, that the sponge has a smooth, porous surface layer. Because of the porosity, this layer is surrounded with liquid, and so is not pressed hard against its mating surface. At the same time it is smooth enough not to become entangled with the similar layer on the other surface of the bearing. The effect of porosity was demonstrated with closed-cell sponge. One of the cover sheets, and the underlying cells, was perforated with a sewing needle. This porous surface was run against glass using soapy water lubrication and showed much less friction (Fig. 1, curve *d*) than the unperforated cover sheet (Fig. 1, curve *c*).

A true animal joint model with two similar surfaces was much more difficult to imitate. It was hard to find a porous material which did not have an enormous friction coefficient when rubbed against itself. I found the best material to be sausage casing made from sheep intestine, lubricated with soapy water. Imitation cartilage was made by stretching this over the cut-open face of closed-cell sponge. Running two 'cartilages' against each other gave the result shown in Fig. 2, curve *a*, where the friction coefficient starts low and rises as seepage occurs. To check that trapped water is necessary, open-cell sponge was substituted for closed-cell sponge in the previous arrangement (Fig. 2, curve *b*). Easy escape of the water quickly raises the friction. Finally, the sponges were replaced by impervious 'Neoprene' sheets. Fig. 2, curve *c*, suggests that hydrostatic lubrication is occurring. This is presumably because the sausage casing itself contains considerable free water.

It should be pointed out that although the reasoning in this communication arrives at the permeable-surface-sponge-backed bearing as a modification of the sponge-type hydrostatic bearing, it could equally well be thought of as a bearing with a thick film of lubricant, where 'weeping' through the porous wall supplies enough liquid to maintain the film.

Weeping bearings give friction coefficients as low as those in animal joints. They can be made by

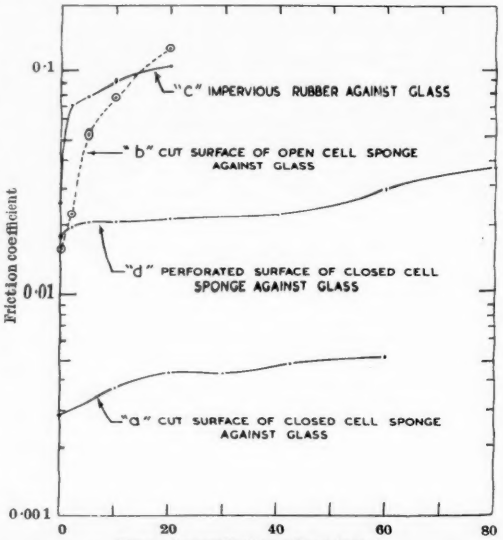

Fig. 1. Friction of various surfaces against glass when lubricated with soapy water

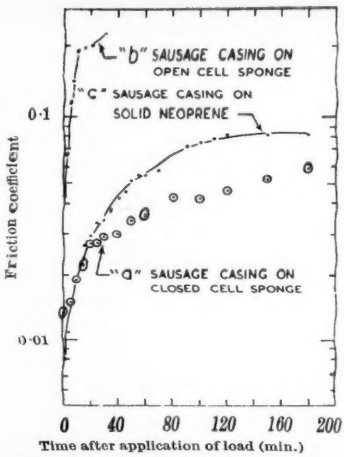

Fig. 2. Friction of various types of surface against themselves when lubricated with soapy water

using surfaces and lubricants which do not give exceptionally low friction under boundary lubrication conditions. (The animal-derived sausage casing used showed entirely ordinary friction coefficients once the excess lubricant had been wrung out.) Animals joints could operate in this way. In the following communication evidence is presented to suggest that they do.

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Experimental Evidence for Weeping Lubrication in Mammalian Joints

FROM existing reports[1] it is clear that the structure and mechanical properties of articular cartilage are just what are required for weeping lubrication to be possible. Its outer surface is formed by a narrow layer of flattened cartilage cells and below this is a relatively acellular zone extending for some hundreds of microns down to the calcified tissue. What cells there are in this zone are arranged in columns, well separated by wide areas of intercellular matrix, which probably has a structure orientated normal to the surface layer. Articular cartilage is easily deformed by pressure but is very resilient, being almost perfectly elastic to intermittent pressures; and it has been suggested that this elasticity is due to exudation and re-absorption of fluid[2]. Thus articular cartilage appears to resemble a rather stiff sponge, with an internal structure which would permit easy expression of fluid up to a smooth, presumably porous, outer surface.

Structural evidence alone is inadequate; so a few simple experiments have been made on articular surfaces from freshly opened joints of a number of mammals. Thin shavings were analysed for sodium and potassium with a flame photometer. The ratio of sodium to potassium found was of the order of 12–15 on a molar basis; so articular cartilage must contain a very high proportion of extracellular fluid, as suggested by histological preparations. Exudation of only a small fraction of this total extracellular fluid would provide an adequate lubricating film. That the superficial layer of flattened cartilage cells is freely permeable to small molecules is easily shown by dropping aqueous solutions of dyes on to a freshly exposed surface. Dyes such as eosin, for example, rapidly penetrate to a depth of at least a hundred microns. Furthermore, if excess dye is washed off, some of that which has penetrated can be re-extracted by pressing filter paper firmly against the articular surface. The pore size in this superficial layer is probably quite small, since a graphite suspension with a particle size of rather less than 1μ did not appear to penetrate.

If an articular surface which has been well dried with filter paper is placed against a glass slide and the point of contact examined through the glass with a microscope, fluid can be seen to exude as pressure is applied. The amount of fluid exuded was estimated by placing a small piece of filter paper of known area on an articular surface and applying pressure for a brief period of time (less than a second). The sodium content of the filter paper was then measured with a flame photometer and the volume of exuded fluid calculated on the assumption that it had the same sodium content as extracellular fluid. Both dry and moist filter paper were used with substantially similar results. When the pressure applied was only sufficient to bring the filter paper into intimate contact with the cartilage, the amount of sodium collected was insignificant. As the pressure was raised, however, the amount collected increased, and for pressures in the range to be expected in normal operation of the joint the volume of fluid exuded was calculated to be sufficient to form a layer 15–35μ thick over the area of contact. Between two articulating surfaces twice as much fluid should be available, which ought to be sufficient for adequate lubrication by the mechanism suggested in the previous communication.

Weeping lubrication could equally well occur where tendons change direction (for example, the patella at the knee joint), for the cartilage surface concerned appears to have the same properties as that in the joint proper.

One possible disadvantage of weeping lubrication is the occurrence of a slow outward seepage of fluid from between the apposed surfaces, which might eventually come into contact. Joints seldom remain in a fixed position for very long when they are bearing a load. Animals which sleep standing up, for example horses, are said always to change their position at least every half an hour, and examples are quoted in the previous communication of model bearings which retain their low friction for at least this length of time. The rate of seepage would be markedly affected by the microstructure of the articular surface, but unfortunately little is known about this.

It has not proved possible to devise a crucial experiment which would prove conclusively whether or not 'weeping' lubrication is an important factor in reducing joint friction. Nevertheless, the evidence put forward here strongly suggests that all the necessary conditions are present; so it would be strange indeed if this type of lubrication did not in fact occur.

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EXPERIMENTS ON THE DEVELOPMENT OF ISOLATED BLASTOMERES OF MOUSE EGGS

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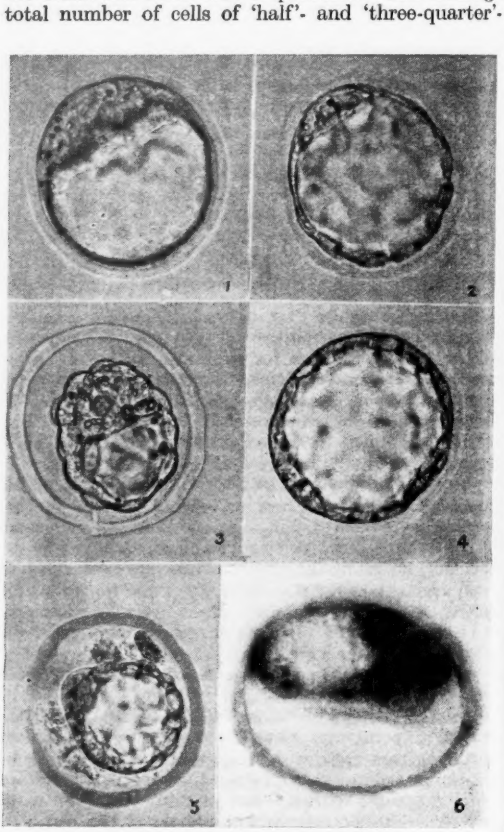
EXPERIMENTAL research on the developmental potency of blastomeres of mammalian eggs has not, so far, advanced beyond the preliminary period. Nicholas and Hall[1] found that development of embryos starts to take place from both separated blastomeres of a 2-cell rat egg. These embryos, although completely normal in structure, did not, however, advance beyond the egg cylinder stage, and underwent resorption before the tenth day of development. Seidel[2] succeeded in obtaining two young rabbits which had developed from 2-cell eggs in which one of the blastomeres had been destroyed by piercing it with a glass needle; he did not carry out any checks of the development during pregnancy. Apart from a short note by Pincus[3], there are no reports in the literature on the development of isolated blastomeres before implantation.

The investigations described below were carried out on mouse eggs, and were aimed at: (1) tracing the entire embryonic development of 'half'-embryos; and, on this basis, (2) defining the regulation capacities of 'half'-blastomeres and of the factors on which they are dependent. Special attention was devoted to the structure of the blastocysts in order to ascertain to what extent the inner cell mass and trophoblast participate in their total mass. Experiments were also carried out on a smaller scale on the development of 4-cell eggs of which one or three blastomeres had been destroyed.

The blastomeres were destroyed under a dissecting microscope by piercing them through the zona pellucida with a glass needle fixed to a micro-manipulator similar to that described by Goldacre[4]. During this operation the eggs were drawn to and held against the mouth of a micro-pipette, with an outer diameter of about 40μ, the suction power of which was regulated by means of the rubber bulb attached to it. All manipulations were carried out in mouse serum diluted 1 : 1 with normal saline. Directly, or a few minutes after piercing the blastomere, it disintegrated totally or partially. Using the technique previously described[5], the eggs were then transferred to the oviducts of the recipients, which had been mated the previous night with vasectomized males. Animals with differing pigmentation were used as donors and recipients for the experiments, which were aimed at obtaining embryos from the second half of pregnancy or young.

Development before implantation. A total of about 100 'half'- and 'three-quarter'-cleaving eggs and blastocysts was obtained. The volume of most 'half'- and 'three-quarter'-blastocysts is similar or only slightly smaller than that of normal blastocysts (compare Fig. 1 with Figs. 2 and 4). Some 'half'-blastocysts were, however, encountered lying loosely within the zona pellucida (Fig. 3). The volume of the inner cell mass of 'half'-blastocysts, treated as the elliptical cap, is subject to wide variations, but in no case does it attain the smallest value found in normal blastocysts. Its average size, as a percentage of the size of inner cell mass of normal 3½-day blastocysts, is 44·5. The volume of the inner cell mass of 'three-quarter'-blastocysts varies within the wide limits of variability observed for 'half'- and normal blastocysts.

After carrying out observations in the living state, the blastocysts were then fixed and mounted *in toto* in order to determine the number of cells composing the inner cell mass and trophoblast. The average total number of cells of 'half'- and 'three-quarter'-



(1) 3½-day normal blastocyst (× 300). (2) 'Half'-blastocyst. Size equal to normal, small inner cell mass (× 300). (3) 'Half'-blastocyst lying loosely within zona pellucida (× 300). (4) 'Half'-blastocyst without differentiated inner cell mass (× 300). (5) 'Quarter'-blastocyst composed of about twenty cells. Most of them form trophoblast (× 300). (6) 'Quarter'-blastocyst composed of only eight cells, six in inner cell mass and two in trophoblast. Stained with hæmatoxylin, mounted *in toto* (× 750)

blastocysts was respectively 68·5 and 68·3, and number of cells of the inner cell mass is 33·0 and 58·4, again as a percentage of the number in normal 3½-day blastocysts.

The numerical ratio between the cells of the inner cell mass and trophoblast varies considerably in the various 'half'- and 'three-quarter'-blastocysts. Among the developing eggs there were several 'half'-blastocysts without a differentiated inner cell mass (Fig. 4), and several morulas in which, despite the aggregation of a large number of cells, the differentiation of the trophoblast had not taken place.

It would seem that my results are connected in a logical manner with those of cytochemical investigations by Dalcq and his co-workers on the development of the eggs of rodents[6–9]. The following facts established by these authors are the most important for the interpretation of present observations: (1) the oocyte and fertilized undivided egg have a bilateral symmetry resulting from the disposition of the two cytoplasmic zones, which differ from each other cytochemically. The cytoplasm of the dorsal and ventral zones passes respectively to the cells of the inner cell mass and the trophoblast. (2) The plane of the first cleavage division has no established relation to the plane of symmetry of the egg.

The variations in the numerical ratio between cells of the inner cell mass and the trophoblast in 'half'-blastocysts, and the variations in the volume of the inner cell mass itself, are presumably the result of variable distribution of the cytoplasm of these zones to each of the 'half'-blastomeres. According to Jones-Seaton[7], there is a tendency in the ova of rats to symmetrical or oblique placing of the plane of the first division. This would explain the fact that the majority of 'half'-blastocysts consist of both the inner cell mass and trophoblast, and that forms having only one of these elements are relatively rarely encountered. As a result of the second cleavage division, the difference between the blastomeres, as regards the character of the cytoplasm forming them, becomes even more emphasized[7,8]. The destruction of a blastomere chiefly composed of cytoplasm intended for the formation of either the inner cell mass or the trophoblast would result in wide variations in the structure of 'three-quarter'-blastocysts.

A single 'quarter'-blastomere is also capable, at least in certain cases, of forming a blastocyst composed of the inner cell mass and trophoblast (Figs. 5 and 6). The degree of participation of these elements in the structure of these two 'quarter'-blastocysts is, however, quite different.

Since the degree of formation of the inner cell mass and trophoblast in 'half-' and 'quarter'-blastocysts is not identical with that in normal blastocysts, the regulating capacities of 'half'- and 'quarter'-blastomeres cannot be considered complete. The fate of the cells which have arisen from a given blastomere must to a large extent be determined by the character of the cytoplasm forming that blastomere. The fundamental factor determining the developmental potency of 'half'-blastomeres in each case would thus be the position of the first cleavage division in relation to the plane of symmetry. The first two 'sister' blastomeres can, but need not necessarily, be characterized by identical capacities for further development.

Development after implantation. Of 175 transplanted 'half'-blastomeres, 54, or 30·8 per cent, were implanted, and 30, or 17·1 per cent, were developing normally at autopsy. In actual fact the capacity of the 'half'-blastocysts to become implanted and continue development is at least twice as great, since only 50 per cent of the transplanted blastomeres are present in the genital tract of the recipients before implantation.

A series of 'half'-embryos on successive days of pregnancy, from the fifth to the fifteenth, was obtained. All the embryos were completely normal in structure. In the period from the fifth to the tenth day their size, calculated by adding up the areas of the sections, does not exceed half the size of the normal control embryos of corresponding age. The statement made by Nicholas and Hall[1] that the size of rat embryos developed from 'half'-blastomeres comes within the limits of variation of the size of normal embryos is therefore somewhat surprising. In the first half of pregnancy, the rate of morphogenesis of 'half'-mouse embryos is in certain cases completely normal, and in others slightly slowed down. Delay in reaching the successive stages never exceeds 24 hr. and most often is considerably less.

Two periods of intensified resorption of 'half'-embryos may be noted—one immediately following implantation, and the other at the beginning of the second half of pregnancy, about the eleventh day. On the eleventh day certain 'half'-embryos reach the stage and size characteristic of normal development; others correspond to tenth-day normal embryos. Dead embryos are also encountered. From the twelfth day on, the number of resorbed embryos increases markedly. On the other hand, all the surviving embryos are already at the same stage of development and of the same size as the normal ones. Rapid increase in the rate of development of 'half'-embryos, which takes place at this time, seems to be caused by two factors: (1) real increase in the rate of growth connected with the beginning of functioning of the chorio-allantoic placenta, (2) apparent hastening of development due to elimination of most retarded embryos by resorption.

Since all the embryos undergo normal development until the eleventh day, and complete regulation may be said to have taken place, resorption must be only the result of disturbance of the normal relations between mother and embryo, connected with considerable retardation in the rate of development.

In view of my observations it seems that resorption of all 'half'-rat embryos during the first half of pregnancy, reported by Nicholas and Hall[1], had been caused to a large extent by improper experimental technique.

Three females gave birth to young which had developed from 'half'-blastomeres (first female, 1 young one after 20 days; second female, 2 young ones after 19 days; third female, 3 young ones after 20 days). These young comprised four females and two males. All the animals were fertile, and each female gave birth to several litters. No abnormalities of the kind described by Seidel[2] or elsewhere were found in the young or in the more advanced embryos.

A full account of this work will be published in *Acta Theriologica*.

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METABOLIC AND CHEMICAL STUDIES OF 'MYLERAN': FORMATION OF 3-HYDROXYTETRAHYDROTHIOPHENE-1,1-DIOXIDE *IN VIVO*, AND REACTIONS WITH THIOLS *IN VITRO*

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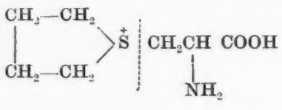
IT has been reported recently[1] that the S-β-alanyltetrahydrothiophenium cation (I) is formed by the reaction of dimethanesulphonyloxybutane ('Myleran') with cysteine. This compound is labile under a variety of conditions, decomposing to tetrahydrothiophene, in which the sulphur atom has been derived from the cysteinyl moiety. Thus treatment with mild alkali (*p*H 8–9), electrolysis of its aqueous solution, or pyrolysis at 140° C. lead to a nearly quantitative conversion into tetrahydrothiophene.

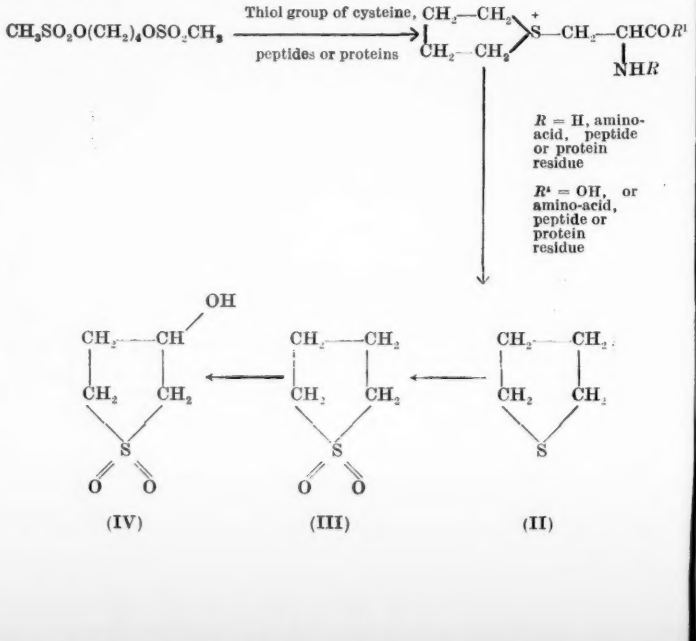


(I)

Since rats which had received injections of either 2,3-^{14}C-'Myleran' (prepared by Dr. P. Brookes) or the corresponding labelled sulphonium compound (I) excreted the same major urinary metabolite, it seemed very likely that intermediate formation of a sulphonium compound of this type occurred also *in vivo* after injection of 'Myleran'. On the basis of the *in vitro* findings which demonstrated the lability of the sulphonium compound and the course of its decomposition, it was suggested that the major urinary metabolite was a derivative of tetrahydrothiophene. This has now been established, since injection into a rat of ^{35}S-tetrahydrothiophene (II) again led to the excretion of only one major metabolite, identical with that formed from 'Myleran' and the sulphonium compound as judged by its chemical properties, and its R_F value in several solvent systems. It was thus further evident that the metabolite contained a sulphur atom. The fact that it failed to give a positive test for sulphur on paper chromatograms suggested that one of the changes which had occurred involved oxidation of the sulphur atom. ^{35}S-Tetrahydrothiophene-1,1-dioxide (III), a likely product of oxidation *in vivo*, was therefore synthesized and shown to be converted in the rat into one major urinary metabolite which differed in R_F value from the parent dioxide, but which was again identical with that formed from 'Myleran', the sulphonium compound, and tetrahydrothiophene.

Possible pathways for the metabolism of tetrahydrothiophene-1,1-dioxide included reduction of the sulphone group, further oxidation, or ring substitution. It seemed improbable that reduction was involved in view of the marked stability of the sulphone group to reduction *in vitro*. Further oxidation of the tetrahydrothiophene-1,1-dioxide could result in unsaturation of the ring, the formation of a ketone, or fission to a di-acid. It was concluded that these changes had not occurred as the metabolite was unaltered by treatment with bromine or warm neutral permanganate, was unreactive towards dinitrophenylhydrazine, and was neutral as shown by passage through columns of ion exchange resins. However, ring substitution by hydroxylation was considered possible, particularly in view of the enhancement of the reactivity of the ring carbon atoms towards nucleophilic reagents by the powerfully negative sulphone group. The presence in the metabolite of one or more hydroxyl groups was demonstrated by its reactivity towards acetyl chloride, or benzoyl chloride in pyridine, leading to the formation of new compounds from which the metabolite could be regenerated by hydrolysis with mineral acid.

Injection of necessarily small quantities of 'Myleran' into a rat had hitherto precluded isolation of the metabolite in a pure state and in quantities sufficient to isolate a derivative; but with the knowledge that tetrahydrothiophene-1,1-dioxide, a non-toxic compound, gave rise to the same major urinary metabolite, this difficulty was overcome. Moreover, it was demonstrated that the same metabolite was excreted by the rabbit after injection of ^{14}C-'Myleran' or ^{35}S-tetrahydrothiophene-1,1-dioxide. Large doses of tetrahydrothiophene-1,1-dioxide with a radioactive carrier were injected into rabbits, and after extraction of the urinary metabolite into chloroform


Thiol group of cysteine,
peptides or proteins
R = H, amino-acid, peptide or protein residue
R¹ = OH, or amino-acid, peptide or protein residue

(IV) (III) (II)

the residual oil was treated with 3,5-dinitrobenzoyl chloride. The resulting ester was recrystallized to constant specific radioactivity from methanol, and formed almost colourless prisms, melting point 195–197° C.

Analysis	$C_{11}H_{10}O_8N_4S$ requires: C = 40·0, H = 3·0, N = 8·5, S = 9·7, m.w. = 330
Found	C = 40·5, H = 3·2, N = 8·3, S = 9·4, m.w. = 340

This same radioactive ester was prepared from the combined chloroform extracts of the urine obtained from two rabbits, one of which had received a small dose of ^{14}C-'Myleran' to act as carrier, and the other a relatively large quantity of tetrahydrothiophene-1,1-dioxide to enable separation of the derivative. It was also obtained from the chloroform extract of urine from rats injected with high doses of the S-β-alanyltetrahydrothiophenium salt (I) containing a radioactive tracer. Each derivative had melting point 195–197° C. alone, and on admixture.

The foregoing experiments indicate that the urinary metabolite formed from 'Myleran' is monohydroxy-tetrahydrothiophene-1,1-dioxide (IV). 2-Hydroxytetrahydrothiophene-1,1-dioxide, prepared via the 2-bromo compound[2] from radioactive tetrahydrothiophene-1,1-dioxide, was shown to possess a different R_F value from the metabolite. However, the 3,5-dinitrobenzoate of an authentic sample of 3-hydroxytetrahydrothiophene-1,1-dioxide (IV) (sulpholanol), kindly supplied to us by Prof. E. Boyland, had melting point 195–197° C. which was undepressed on admixture with the 3,5-dinitrobenzoate formed from the 'Myleran' metabolite.

The metabolism of 'Myleran' in the rat and the rabbit may be represented in the accompanying scheme.

In connexion with the possible mode of action of 'Myleran' it was of interest to determine whether the tetrahydrothiophene formed from the drug *in vivo* could have been derived by reaction with the thiol group of peptides such as glutathione, or with larger molecules such as proteins. It has been shown that 'Myleran' reacts smoothly with the thiol group of glutathione in an alkaline medium forming tetrahydrothiophene, characterized as its mercurichloride. Similarly, thiol-containing proteins such as denatured egg albumin and reduced keratin have been shown to yield tetrahydrothiophene when treated with 'Myleran' in an alkaline medium, indicating that sulphonium ion formation is not restricted to compounds of low molecular weight.

This novel 'dethiolation reaction' provides additional evidence to the *in vivo* findings in support of the view that reactions of this type may be responsible for some of the diverse pharmacological properties of the bifunctional alkylating agents. Sulphonium ion formation might lead to a modification of the function of certain proteins, but in addition dethiolation could conceivably have more far-reaching effects by actually altering the sequence of amino-acids in a peptide or protein chain. It is hoped that work on these aspects may be continued.

Full details of this work will be published elsewhere.

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We wish to thank Prof. A. Haddow and Dr. L. A. Elson for their interest in the work, and to record our appreciation for helpful discussion with many of our colleagues. We gratefully acknowledge technical assistance from Miss M. Morton.

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REGISTRATION OF THE SPERMICIDAL EFFECTS OF DIOCTYL SODIUM SULPHOSUCCINATE

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STUDIES of the kinetics of spermicidal effects are not possible without a method for the estimation of the proportion of living spermatozoa at a given time. This implies that the time required for this estimation has to be very short in relation to the period of time during which the process is to be studied. By working with dark-field illumination, and using comparatively long photographic exposures, different pictures of the lucid spermatozoa, either immobile or in different kinds of movements, are obtained.

For the present investigation bull spermatozoa were chiefly used. Some experiments, however, were performed with human sperm cells, with identical results. A buffered egg-yolk extract was used as an optically suitable diluent for the semen and the spermicidal substance[1]. The optical equipment consists of a Zeiss microscope with dark-field condenser, heating stage, plane-apochromatic objective (× 10), compensating plane eyepiece (× 8), and a Zeiss carbon arc-lamp for microscope use. The microscope carries a Leica camera, somewhat modified in order to facilitate a fast winding-on of the film. Using the Kodak film 'Tri-*X*', 1·0 sec. is found to be an appropriate time of exposure. Prints of the negatives are enlarged five times. The final linear enlargement is thus 400 times.

For each determination of the percentage of mobile spermatozoa two exposures are made with an interval of 1·0 sec. On the first picture the moving spermatozoa leave tracks according to their different ways of moving. A comparison between the first and the second picture in a pair reveals whether doubtful cases depend upon passive motions induced by other spermatozoa or upon active movements. It also renders possible classification of cells disappearing by swimming in the direction of the optical axes as moving.

When performed with all precautions two counts on the same sample generally do not differ by more than 1·5 per cent. All the operations described below are performed at 37° C. with materials which have been preheated to this temperature. Of the semen, diluted to give about 20,000 spermatozoa/μl., that is,

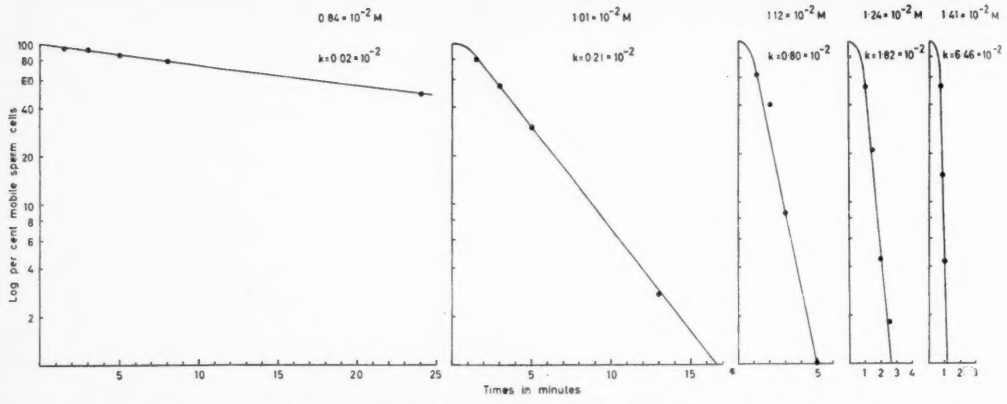

Fig. 1. Semi-log plot of percentage of mobile sperm cells versus time in minutes at different concentrations of dioctyl sodium sulphosuccinate. k, Apparent death-rate constant

twice the final density wanted, 0·5 ml. is brought into a thick-walled test-tube provided with a rod-shaped magnetic stirrer. The tube is placed in a rotating (180 r.p.m.) magnetic field and 0·5 ml. of a solution of the spermicidal substance in diluent (twice the concentration to be studied) is added, all statements as to time being related to this moment as zero. About 10 μl. of this mixture are put on a slide, and a coverglass provided with a fine rim of 'Vaseline' along its edges is placed on the drop in such a way that no air bubbles are left between the two glasses. The preparation is thus protected from evaporation, aerobiosis being, however, permitted only for limited periods of time. The first exposures are made after 60 and 61 sec., and are followed by two exposures every fifteenth and thirtieth second.

A stock solution of dioctyl sodium sulphosuccinate in acetone (0·45 M, 20 per cent) is prepared and added to the diluent. The highest final concentration of acetone in our experiments was about 5 per cent. In control experiments this concentration had no effect on the percentage of mobile spermatozoa for periods of up to 2 hr.

Concentrations of dioctyl sodium sulphosuccinate ranging from $0{\cdot}84 \times 10^{-2}$ M to $1{\cdot}41 \times 10^{-2}$ M (0·38 per cent to 0·63 per cent) give exponential death-rate curves that are more easily read in logarithmic form (Fig. 1). As in corresponding experiments with bactericidals[2] these straight curves permit the calculation of 'apparent death-rate constants'. The analytical plotting of the relation between concentration of dioctyl sodium sulphosuccinate and the apparent death-rate constants is seen in Fig. 2. According to the theoretical analyses given by Johnson, Eyring and Polissar[3], these curves may give considerable information about the mechanism involved. Tentatively we have transformed their expression:

$$\pi = 1 - p^m = 1 - (1 - e^{-kt})^m$$

into

$$\log (1 - \pi) = m \log (1 - e^{-kt})$$

and plotted $\log (1 - \pi)$ against $\log (1 - e^{kt})$ choosing such a value of k as to give a straight line (Fig. 3). This implies identical values of m and n. With decreasing concentrations of dioctyl sodium sulphosuccinate both k and n decrease. Log n plotted against log concentration gives a linear relationship. As the concentration of the spermicide is large it remains in excess, and may thus be considered

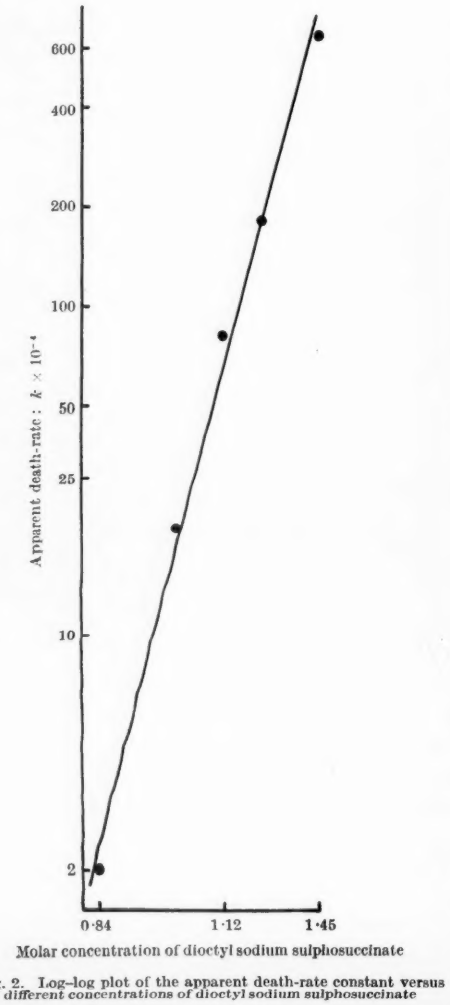

Fig. 2. Log-log plot of the apparent death-rate constant versus different concentrations of dioctyl sodium sulphosuccinate

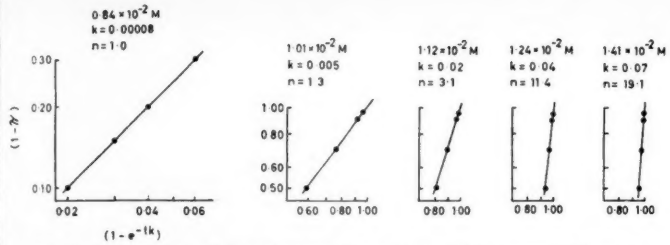

Fig. 3. Log–log plot of $(1 - \pi)$ versus $(1 - e^{-kt})$ at different concentrations of dioctyl sodium sulphosuccinate

constant[2]. According to Johnson, Eyring and Polissar n may thus represent the number of molecules of dioctyl sodium sulphosuccinate combining with each molecule of protein in the cells. Occasionally curves relating log surviving sperm cells to time obtained with low concentrations ($0{\cdot}84 \times 10^{-2}$ *M*) of dioctyl sodium sulphosuccinate show a sharp break, the death-rate suddenly decreasing. This observation will be further analysed. The effects on the relationships described of anaerobiosis, temperature and ageing of the cells are being studied.

Corresponding results have been obtained with chloramin-*T* and hydrogen peroxide.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND HUMAN ERYTHROCYTES

Characteristics of Glucose-6-Phosphate Dehydrogenase from Normal and Primaquine-sensitive Erythrocytes

RECENT developments have emphasized the fact that genes may express themselves in terms of qualitative alterations of such macromolecules as human hæmoglobins[1] and blood-group substances[2]. Whether or not such qualitative differences at the level of the enzyme usually account for the inherited enzymic deficiencies is not known, but some instances of qualitative alterations in enzymes have been reported. Immunochemical evidence[3] exists for an inactive enzyme-like molecule in mutants lacking tryptophan-synthetase. Qualitative differences in glutamic dehydrogenase have been found in mutants of *Neurospora*[4]. An opportunity exists to explore this possibility further in the case of primaquine-sensitive hæmolytic anæmia[5], an inherited defect in which the erythrocytes of affected hemizygous or homozygous persons have only 5–20 per cent of the normal activity of glucose-6-phosphate dehydrogenase.

In order to investigate possible qualitative or quantitative differences between the glucose-6-phosphate dehydrogenase in primaquine-sensitive and normal erythrocytes, a method of partially purifying the enzyme has been developed[6,7] and preliminary comparative studies have been made. The partially purified preparation is hæmoglobin-free and represents a 60- to 80-fold purification of glucose-6-phosphate dehydrogenase in 20–50 per cent yield, with little or no contamination with 6-phosphogluconic dehydrogenase. The enzyme was assayed in final concentrations of 0·1 *M tris* buffer, *p*H 8·0, 0·01 *M* magnesium chloride, 2×10^{-4} *M* triphosphopyridine nucleotide, and $6{\cdot}0 \times 10^{-4}$ *M* glucose-6-phosphate, by observing the increase in optical density at 340 mμ or by measuring the 450 mμ fluorescence in a photofluorometer, using 360 mμ exciting light. Within an accuracy of ± 5–20 per cent, the preparation from both a primaquine-sensitive male and a normal male control had Michaelis constants of $2{\cdot}1 \times 10^{-6}$, $3{\cdot}9 \times 10^{-5}$, and $6{\cdot}9 \times 10^{-4}$ *M* for triphosphopyridine nucleotide, glucose-6-phosphate, and 2-deoxyglucose-6-phosphate, respectively. Both utilized the last substrate at 9 per cent of the maximum rate for glucose-6-phosphate. In a mixed buffer which was 0·05 *M* each in phosphate, *tris* and glycine, both preparations had the same *p*H-optimum curve from *p*H 6·0 to 10·0. Heat of activation (20–40° C.) was found to be $9{\cdot}5 \times 10^3$ cal./mole for both preparations. Similar movement on anion-exchange column chromatography has been observed for the enzyme from both sources[7]. The percentage yield of activity for the enzyme from sensitive cells remained approximately the same as that for normal cells throughout the partial purification and all experiments. Similar labilities and the stabilizing effect of triphosphopyridine nucleotide have been reported[6]. The latter may be related to the inactivation of this enzyme by erythrocytic stroma, which has been observed by others (ref. 8 and following communication).

Because of the many identical catalytic parameters, it seems unlikely at the present time that the greatly reduced activity of erythrocytic glucose-6-phosphate dehydrogenase in these persons is due to a qualitative difference at the catalytic site of the enzyme, if a qualitative difference exists at all. We are left, therefore, with the necessity of considering a gene which manifests itself through decreasing the rate of synthesis or increasing the rate of inactivation of this enzyme. The fact that the 80-fold purified preparation can be far more extensively purified indicates that the glucose-6-phosphate dehydrogenase constitutes only a very minute portion of the hæmoglobin-free proteins and emphasizes the necessity and opportunity for more definitive comparisons of relatively pure preparations of glucose-6-phosphate dehydrogenase. Some differences in labilities[8,9] and *p*H optima[9] between this enzyme in normal and primaquine-sensitive hæmolysates have been reported. In view of the extensive contaminations with other proteins and the stabilizing effect of small amounts of various substances[6], caution should be taken in drawing inferences as to molecular differences based on differences in lability of the glucose-6-phosphate dehydrogenase between normal and prim-

aquine-sensitive hæmolysates and crude preparations.

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Mechanism of Inactivation of Glucose-6-phosphate Dehydrogenase in Human Erythrocytes

In the course of investigation of primaquine-sensitive hæmolysis we found that glucose-6-phosphate dehydrogenase is inactivated by incubation with stromata in hæmolysates of primaquine-sensitive and non-sensitive erythrocytes alike[1,2]. Purified hæmoglobin-free glucose-6-phosphate dehydrogenase from both kinds of erythrocytes appears qualitatively identical and is highly stabilized by triphosphopyridine nucleotide[3].

We wish to report that in hæmolysates of both types of cells 'Norit' inactivates glucose-6-phosphate dehydrogenase by adsorption of pyridine nucleotides[4] as do stromata by pyridine nucleotidase activity. Inactivation of glucose-6-phosphate dehydrogenase by stromata is prevented by triphosphopyridine nucleotide, diphosphopyridine nucleotide and nicotinamide. Inactivation by 'Norit' in stroma-free hæmolysates is prevented by tri- and di-phosphopyridine nucleotide but not by nicotinamide. Dialysed stroma-free hæmolysates retain bound coenzyme and, in them, glucose-6-phosphate dehydrogenase is not inactivated by incubation unless stromata or 'Norit' is added. Glucose-6-phosphate dehydrogenase inactivation and removal of bound coenzyme occur concomitantly during incubation with stromata or 'Norit'.

Human erythrocytes washed with cold 0·145 *M* sodium chloride were hæmolysed by rapid freezing and thawing. The hæmolysate was diluted with four or five volumes of cold water; the stromata were removed by centrifuging for 60 min. at 28,000*g*, 0° C. either before or after incubation. After dialysis (usually overnight) in 0·067 *M* phosphate buffer, *p*H 7·4, the hæmolysates were assayed for glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase activity by a modification of the coupled reactions with glutathione reductase previously described[1]. In a final volume of 7·0 ml. the complete reaction mixtures contained: (1) *tris*-hydroxymethylaminomethane, 5×10^{-4} *M*, adjusted to *p*H 7·4 with hydrochloric acid; (2) ethylenediamine tetraacetic acid adjusted to *p*H 7·4 with sodium hydroxide, 5×10^{-5} *M*; (3) triphosphopyridine nucleotide (Sigma), 7×10^{-6} *M*; (4) oxidized glutathione, GSSG (Schwarz), 5×10^{-6} *M*; (5) glucose-6-phosphate (Sigma), 4×10^{-6} *M* for glucose-6-phosphate dehydrogenase; or (6) 6-phosphogluconate (Sigma), 4×10^{-6} *M* for 6-phosphogluconic dehydrogenase; and (7) varying amounts of hæmolysate as shown in Fig. 1. Reactions were run for 15 min. at 37° C., *p*H 7·4.

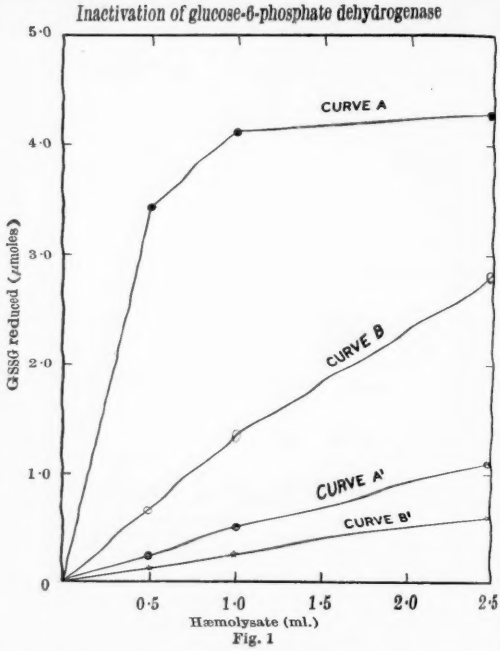

Fig. 1

Fig. 1 shows representative results from a male non-sensitive (*A*, *A'*) and a male sensitive individual (*B*, *B'*). Curves *A* and *B* represent activity of glucose-6-phosphate dehydrogenase in dialysed hæmolysates from which stromata had been removed immediately after hæmolysis. Curves *A'* and *B'* show activity of glucose-6-phosphate dehydrogenase, after incubation of hæmolysate from non-sensitive cells for 1 hr. at 45° C. and from sensitive cells for 1 hr. at 37° C., prior to removal of stromata. Addition of tri- or di-phosphopyridine nucleotide (10^{-4} *M*) to these hæmolysates before incubation provided 100 and 73 per cent protection, respectively, based on approximate integration of the curves obtained. Nicotinamide (5×10^{-3} *M*) provided protection ranging from 15 to 60 per cent in non-sensitive and 60 to 100 per cent in sensitive hæmolysates.

Similar incubation of doubly centrifuged stroma-free hæmolysates results in little or no inactivation either before or after dialysis. However, glucose-6-phosphate dehydrogenase is inactivated in stroma-free hæmolysate by incubation with acid-washed 'Norit' (2·5–30 mgm. per ml.) or by incubation after removal of 'Norit'. Triphosphopyridine nucleotide completely protected glucose-6-phosphate dehydrogenase in stroma-free hæmolysates incubated after removal of 'Norit'. Diphosphopyridine nucleotide protected it in non-sensitive hæmolysates by 47 per cent and in sensitive hæmolysates by 91 per cent. However, nicotinamide gave no protection, suggesting that its effect occurs by inhibition of stroma factor, whereas tri- and di-phosphopyridine nucleotide stabilize the enzyme itself.

Glutathione reductase and 6-phosphogluconic dehydrogenase in hæmolysates, unlike glucose-6-phosphate dehydrogenase, remain active even after incubation with stromata or 'Norit'. This allows preliminary investigation of the binding of triphosphopyridine nucleotide to glucose-6-phosphate dehydrogenase because the former is also the coenzyme of 6-phosphogluconic dehydrogenase. Stroma-free dialysed hæmolysates retain sufficient triphosphopyridine nucleotide for the hæmolysate to reduce GSSG in 1 hr. at 37° C. when 6-phosphogluconate, but not triphosphopyridine nucleotide, is added to the reaction mixture. However, under the same conditions, GSSG is not reduced after incubation with stromata or 'Norit'. (Triphosphopyridine nucleotide is retained in hæmolysate which has been dialysed for as long as 42 hr.)

The inactivation of glucose-6-phosphate dehydrogenase and loss of triphosphopyridine nucleotide remaining in dialysed hæmolysates occur together, suggesting that the non-dialysed fraction of triphosphopyridine nucleotide is bound to glucose-6-phosphate dehydrogenase and that this is active only when bound with its coenzyme. The 6-phosphogluconic dehydrogenase does not bind triphosphopyridine nucleotide or require it for stabilization.

Intact human erythrocytes have pyridine nucleotidase activity, both on the surface and within the cell: the surface activity is demonstrated by the inactivation of glucose-6-phosphate dehydrogenase in isotonic hæmolysates during incubation with whole human erythrocytes; the intracellular effect is shown by a loss of glucose-6-phosphate dehydrogenase activity and bound triphosphopyridine nucleotide in erythrocytes incubated in isotonic saline for 2 hr. at 45° C. Although no loss of glucose-6-phosphate dehydrogenase activity occurred during storage of non-sensitive blood, gradual loss of glucose-6-phosphate dehydrogenase activity in sensitive blood during four weeks of storage in acid–citrate–dextrose solution also suggests an intracellular action of pyridine nucleotidase.

These results show that triphosphopyridine nucleotide stabilizes glucose-6-phosphate dehydrogenase in hæmolysates as well as in partially purified preparations and confirm the suggestion that this stabilization may be related to the inactivation of this enzyme by erythrocytic stroma (ref. 5 and previous communication). Partial stabilization of glucose-6-phosphate dehydrogenase by nicotinamide is indirect, by protection of the pyridine nucleotides from pyridine nucleotidase activity of stromata. Stabilization by diphosphopyridine nucleotide may also be indirect, by its enzymatic conversion to triphosphopyridine nucleotide, since diphosphopyridine nucleotide does not stabilize glucose-6-phosphate dehydrogenase of partially purified preparations[5]. Nevertheless, protection of glucose-6-phosphate dehydrogenase of stroma-free hæmolysates by di- as well as triphosphopyridine nucleotide indicates that the former can help to stabilize glucose-6-phosphate dehydrogenase in human erythrocytes, even though it is not a coenzyme for it. The key to the mechanism of primaquine hæmolysis, and possibly of cellular ageing, may be related to these stabilizing processes.

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X-RAY DAMAGE AND RECOVERY IN MAMMALIAN CELLS IN CULTURE

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As measured by the ability to sustain unlimited proliferation, the X-ray sensitivity of microorganisms has been generally observed to be much greater than the sensitivity of important macromolecules. This, in itself, has been an important reason for associating the lethal effect of X-rays with the genetic apparatus of the cell. Puck and Marcus's[1] observation that mammalian cells in tissue culture were even more sensitive—10–100 times or more—than bacteria or yeasts further reinforced this view and led to their very reasonable proposal that the sensitive sites in mammalian cells are the chromosomes.

If the functional integrity of the genetic apparatus is required for viability, since the survival curves of most somatic cells are sigmoid (or multihit), it might be expected that survivors after X-irradiation would be more sensitive to subsequent exposure than the parental population. This follows from the fact that multihitness implies a threshold type of response (which means damage must be accumulated before an effect is observed) and hence that surviving cells accumulated a sublethal amount of damage.

We have investigated the question of the presence of heritable damage in two cell lines of the Chinese hamster, *Cricetulus griseus*[2] (clone *A* ovarian tissue and strain *V* female lung tissue), propagated in tissue culture. We have found that essentially all the survivors after X-irradiation did not display heritable damage, as would be evidenced by their radiosensitivity, but rather that they repaired their accumulated damage before their first division after irradiation.

Our growth medium bears the designation *HU*-15. It consists of Eagles's amino-acids and vitamins[3] with glutamine at a concentration of 1 m*M*; 4 per

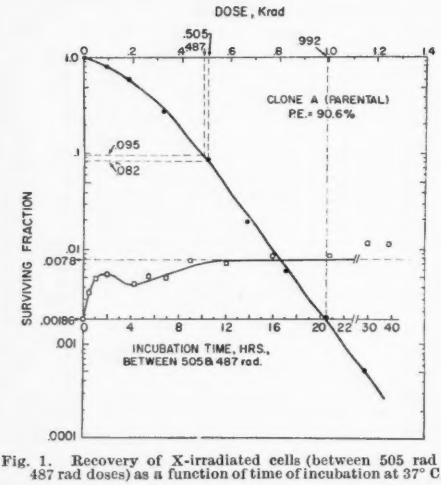

Fig. 1. Recovery of X-irradiated cells (between 505 rad and 487 rad doses) as a function of time of incubation at 37° C.

cent Earle's *NCTC*-109 [4]; Puck's[5] saline *F* with the calcium content increased 6·5 times; and 15 per cent undialysed fœtal calf serum (Colorado Serum Co., Denver). Both cell lines grow in this medium with a doubling time of about 12 hr.

Our X-ray source consisted of a Machlett *OEG*-60 tube powered by a full-wave rectified, 55 kV. supply. The tube was operated at 12 m.amp. with 0·175 mm. aluminium filtration; absorbed dose-rate 720 rads/min. After the attachment of cells in 9-cm. Petri dishes, the plate covers and growth medium were removed and the cells were irradiated at room temperature in a humidified atmosphere of 2 per cent carbon dioxide in air. Surviving clones were stained and counted after 12–18 days incubation at 37° C. in a 2 per cent carbon dioxide incubator. Identification of abortive colonies was facilitated by the use of a projection technique, although our results are essentially the same whether or not abortive colonies are included. Plating efficiencies in most of our experiments were about 70 per cent; essentially the same results were obtained, however, in experiments having plating efficiencies from 10 to 90 per cent.

In discussing the observations, a multihit model will be assumed for simplicity although our conclusions apply equally as well to sigmoid or threshold type survival curves in general. For lag-phase cells, clone *A* was found to display hitness numbers of 4–5 and clone *V* 6–7.

X-ray dose fractionation was employed to test for repair of accumulated damage. Fig. 1 shows a survival curve for single clone *A* cells trypsonized and plated 2 hr. before exposure. (Standard errors are indicated where larger than the plotted points.) In addition, the lower portion of the figure shows a recovery curve for cells which had received a first dose, 505 rads, followed by incubation at 37° C. for various periods of time before receiving a second dose of 487 rads.

If there had been no recovery between the exposures, the two doses would have been completely additive, and the survival after a total of 992 rads would have been 0·0019. Alternatively, if there had been complete recovery between doses, the survival to the first and second doses would have been 0·082 and 0·095, respectively. The product of these latter values is 0·0078, which represents the survival corresponding to complete recovery between doses. The points to be noted are: (1) as a function of time at 37° C., the cells recover in a manner which may involve repair of sites as well as fluctuations in sensitivity; (2) for clone *A* cells, recovery appears to be complete by about 10 hr. and constant until about 25 hr.; and (3) the survival rises above 0·0078 after about 25 hr., which probably represents the effect of cellular multiplicity on survival concomitant with the onset of post-irradiation division.

The likelihood of the last point was arrived at by two types of measurements. First, we showed that the 'principle of cellular multiplicity' holds for these cells. That is, the surviving fraction of colonies containing more than one cell is shifted upward by an amount governed by the average cellular multiplicity providing that each cell in the colony has the same average sensitivity and that it must be inactivated independently to suppress post-irradiation colony formation. Secondly, an estimate of division delay following a first dose of 505 rads was made by comparing the clonal growth of irradiated cells with unirradiated controls. The comparison was made after clones had reached a size, about 100 cells per clone, which permitted an unambiguous identification of such clones as survivors. In agreement with the recovery curve in Fig. 1, the latter measurement indicated a division delay of about 30 hr.

In addition to the preceding, we have also shown that recovery can take place at room temperature unaccompanied by division in the control population.

To verify that the plateau region in the recovery curve of Fig. 1 represents complete repair of the accumulated damage resulting from the first dose, the survival curve was repeated after 505 rads followed by 18·1 hr. at 37° C. In Fig. 2, the non-fractionated survival curve was re-drawn starting from the survival corresponding to a first dose of 505 rads. The figure displays an excellent fit of the re-drawn curve to the observed points and shows that, in the exponential regions of both curves, maximum recovery shifts the survival upward by a factor equal to the hitness number. It also follows from Fig. 2 that, at least in the region of full recovery, synergism between the first and second doses is probably absent and therefore the second dose measures the degree of repair of damage resulting from the first dose.

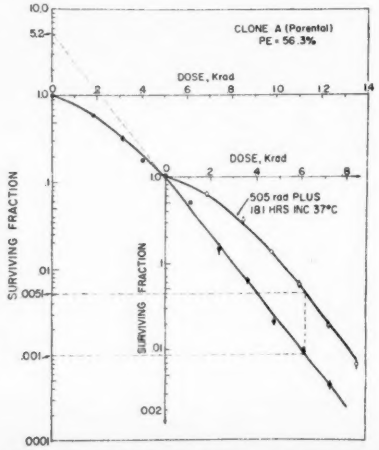

Fig. 2. Full recovery after a dose of 505 rads followed by 18·1 hr. at 37° C. as evidenced by the repetition of the non-fractionated survival curve

The preceding figures are part of a comprehensive study which will be reported in detail elsewhere. To the extent that these and the results to be reported are typical for somatic cells in general, it should be noted that: (1) The vast majority of surviving cells completely repair their accumulated damage before their first division post-irradiation. This means that if the hitness number is n, in the exponential region, survivors undo the effects of a maximum number of hits which is $n-1$. (2) The kinetics of recovery depend on the physiological state of the cells and/or can be caused to appear to undergo large oscillations depending on the recovery medium. These apparent oscillations may result from the combined effects of changes in sensitivity and repair of inactivated sites. (3) Although there are important quantitative differences, log-phase cells respond similarly. (4) A cell can undergo repeated cycles of damage and repair with no apparent attenuation of the repair process(es).

There are several contexts in which these findings are of interest. If the chromosomes are the X-ray sensitive sites and chromosome breaks are the hits leading to lethality, then some new properties of restitution must be considered. First, restitution goes to completion in surviving cells. Secondly, the cell's ability to restitute breaks remains unimpaired after repeated doses. In view of the preceding, Puck's[6] report of a high yield of mutant characteristics in the progeny of cells surviving 5–7 mean lethal doses may be applicable to the material he was using; may be evidence of a radiation-induced chromosomal lability which is expressed after recovery and during clonal growth; may imply that mutation production and lethality are not, in general, closely connected; or may indicate that the chromosomes are not the primary sensitive sites related to viability.

Another area in which these results may apply is in connexion with tumour therapy. Treatment protocols involving fractionation are common, permitting, in general, ample time between treatments for considerable if not complete recovery. Even for situations in which the hitness number may only be 2, a simple calculation reveals that, if recovery is not duly accounted for, the survival using fractionation can be higher than expected by several orders of magnitude. Of course, tissue recovery in a general sense has been recognized by radiation therapists for a long time. These results, however, provide a cellular basis for this phenomenon and lend specific direction to the research that should be undertaken both to take advantage of, as well as to control, this effect.

Additional experiments are planned (or in progress) to examine the influence of dose-rate on survival, and the biochemical and cytogenetic aspects of recovery.

We are indebted to Dr. T. T. Puck for a sample of his clone *A*, ovarian tissue, which has been propagated in our laboratory without recloning since August 1958; to Dr. Denys Ford for his *V* strain, female lung tissue, which was recloned in December 1958; and to Dr. George Yerganian, who supplied the Chinese hamsters for the original explants.

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TALLOWY DISCOLORATION IN CHEDDAR CHEESE

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Fat Oxidation and Trace Metals in Cheese

A SERIOUS fault in mature Cheddar cheese is the gradual appearance of bleached areas with a tallowy flavour, aptly described as 'tallowy discoloration' or 'white streak'. The erratic incidence of this defect has for long hindered efforts to find the prime cause. In seeking the cause, most of the chemical features examined showed little, if any, difference between normal and tallowy portions, apart from the oxidized condition of the latter. The most striking differences were found in the disposition of trace metals. As compared with adjacent normal cheese the copper content of the centre of tallowy portions was always much lower, often as little as a third; but the iron content was always higher, usually by about a half. Experiments with threefold added copper did not affect the incidence of the tallowy defect, nor did added iron salts.

When normal cheese curd was treated with an excess of warm 5 per cent iron-free brine, the portion that dissolved contained more iron than the undissolved cheese, which was shown to lose a corresponding quantity of iron. Moreover, treatment for a shorter period so that less cheese dissolved, resulted in a higher iron content of the dissolved cheese. This indicated the presence of an iron compound more soluble than the cheese, and also suggested a probable connexion between the salting process in cheese-making and the appearance of tallowiness.

The degree of oxidation of iron in cheese was also studied. Practically no ferrous iron could be extracted from young cheese; but the amount extractable increased with ageing at variable rates, some cheese yielding very little at maturity. Cheese tending to tallowiness had increased ferrous iron content, while the actual tallowy seams had the highest ferrous content. This occurred in seams where oxidation was most advanced, as shown by high peroxide values and also by oxidation of the sulphydryl groups of the protein. These results now indicate that an important part is played by an iron complex. The very low copper content at the centre of tallowy seams may be an effect of oxidation of a copper–sulphydryl compound similar to that demonstrated by Stricks and Kolthoff[1].

Most of these investigations were carried out during a period of several years. In view of the recent finding by Rammell (following communication) that hæmatin compounds can cause tallowy discoloration, they support the view that these or similar iron complexes

have an important influence in causing tallowy discoloration.

F. Bishop

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Fat Oxidation and Hæmatin Systems in Cheese

Much work has been done in New Zealand on a defect of Cheddar cheese characterized by a coupled oxidation of the fat and carotene. The defect, commonly known as 'tallowy discoloration' or 'white streak', is seen in the interior of the cheese as bleached areas having a tallowy flavour. These bleached areas are invariably associated with cracks in the body of the cheese. The defect usually occurs only after at least 16–20 weeks storage at low temperatures (42–44° F.). Previous attempts to find the major pre-disposing cause of this defect have not been completely successful.

As I believe the defect to be similar in several ways to oxidative changes described by Tappel[1], attempts were made to show the presence of a fat-oxidizing system in cheese, using methods similar to those of Blain and Todd[2]. Such a system was found to be present in some cheese. As a result of these findings, cheese was made in which sterile, defibrinated cow's blood was added to the cheese milk. The experimental and control cheese were stored in a controlled curing room at about 55° F. for 6 weeks. Sample plugs of the cheese were then stored in the presence of air, at 32–34° F. for 4 weeks. After this time, the cheese containing the highest amount of blood (0·02 per cent in the cheese milk) had developed typical tallowy discoloration. The control cheese containing no added blood had not developed the defect; it was of normal flavour and colour.

On obtaining these results, four of the original experimental and control cheeses were cut and examined. These cheeses had been at 55° F. for 2 weeks followed by 9½ weeks at 42–44° F.—a total of 11½ weeks. Tallowy discoloration was seen in the two experimental cheeses, being more extensive in the cheese containing the higher amount of blood. The two control cheeses showed no sign of the defect.

The possibility of abnormal amounts of blood in the milk being the prime cause of tallowy discoloration must now be considered. It has been shown that, during manufacture of experimental cheese, most of any red blood cells added to the milk become concentrated within the cheese curd. This concentration is further modified by the addition of salt to the curd, resulting in a partial hæmolysis of the red blood cells. Higher concentrations of salt would then be expected to reduce tallowy discoloration. The effect of added salt provides a possible partial explanation of the results obtained by Bishop (preceding communication).

Full details of these and related experiments will be offered for publication elsewhere.

C. G. Rammell

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NON-INVERTED VERSUS INVERTED PLOTS IN ENZYME KINETICS

It was first shown, but not published, by Woolf (see ref. 1) that the Michaelis–Menten equation $v = V_m/(1 + K_M/S)$, relating the initial reaction-rate (v) of an enzymic reaction to the substrate concentration (S) can be written in three linear forms. The experimental (apparent) kinetic constants V_m (v, when $S \to \infty$) and K_M (S, when $v = V_m/2$) can be obtained from plots of the variables $1/v$ versus $1/S$, S/v versus S or v versus v/S. Since its first application by Lineweaver and Burk[2], the $1/v$ versus $1/S$ plot has been used most commonly.

However, it has been pointed out on several occasions (see ref. 3), that the v versus v/S plot, non-inverted with respect to v, has advantages over the two inverted plots, partly because it is less apt to obscure deviations from linearity. With respect to similar plots for determining the number of molecules of a small molecular compound bound to a protein, Scatchard[4] states: ". . . this [inverted plot] has the disadvantage of concealing deviations from the ideal laws and of tempting straight lines where there should be curvature".

These considerations apply also to the determination of kinetic constants from enzymic reaction-rates at constant substrate concentration and varying concentrations of an inhibitor or activator (see ref. 5).

Despite the drawbacks it has remained customary, at least in enzyme kinetics, to use inverted plots in the evaluation of results. Even in a recent and authoritative text-book on enzymology[6] that treats these graphical procedures extensively, the advantages of the v versus v/S plot have not been fully recognized. For these reasons some further comment on this matter seems to be appropriate.

In an acid-base titration, or in the demonstration of the influence of the hydrogen ion concentration on the rate of an enzymic reaction, unwieldy graphs would be obtained if instead of pH the hydrogen ion concentration were used as one of the co-ordinates. This applies also to the influence of the substrate concentration on the rate of an enzymic reaction, and accordingly plots of v versus pS have been used in enzyme kinetics. The inflexion point of the theoretical sigmoid curve corresponds to pK_M.

Like other titrations, the enzyme–substrate 'titration' should be carried out over a range that includes substrate concentrations above as well as below this inflexion ('halfway') point. An ideal range is that of about one pS unit below to one pS unit above this point, that is, from about $0{\cdot}1$ to $10\ K_M$[5,7], representing roughly the range of 10–90 per cent 'saturation' of enzyme with substrate. However, such a (semi) logarithmic plot is still of little value for the estimation of the kinetic constants because it is not linear. On this basis, the advantage of the v versus v/S plot becomes immediately apparent. It can be seen in Fig. 1 that for almost 80 per cent of its course this plot nearly coincides with the v versus pS plot. It has the additional advantage over the latter of being linear, which allows V_m to be determined by extrapolation to $S \to \infty$ (intercept with the ordinate). The (negative) slope is equal to K_M.

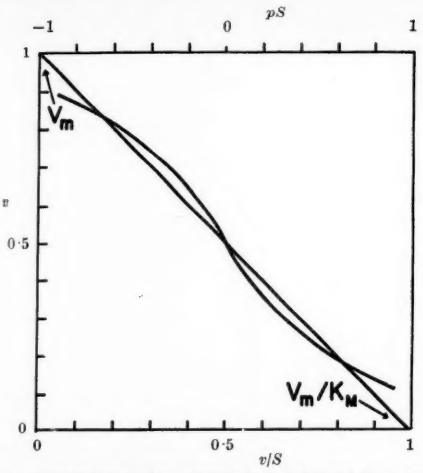

Fig. 1. Comparison of the v versus pS (sigmoid) and the v versus v/S plot (straight line). V_m has been taken as unity for the reaction-rate (v) and K_M as unity for the substrate concentration (S)

If, in the ideal region, substrate concentrations are applied that correspond to about equal increments in pS, a procedure that has been found practicable in many cases (for examples see ref. 8), the reaction-rates actually measured also change by about equal increments and are equally distributed over the v versus v/S plot. This is not the case with the other two plots when equal increments of $1/S$ or S are taken. In this connexion a few comments on statements made by Dixon and Webb (ref. 6, pp. 21–22) are in order. These statements are: ". . . the experimenter is free to choose such substrate concentrations as will give the best distribution of points (one very rarely works at equal increments of v, indeed it is hardly practicable to do so)" and ". . . it is a positive advantage to have most of the points concentrated near the left hand side in [the two inverted] methods, since it is this part of the graph which is most important for determining K_M". When, in the inverted plots, the substrate concentrations are chosen so as to give equal distribution of points, one finds that the corresponding changes in rate decrease progressively at one end of the curve until they become too small to be measured accurately. On the other end of the curve, the increments in v become unnecessarily large. This is demonstrated by Figs. 2*A* and 2*B* for the case of the $1/v$ versus $1/S$ plot. Fig. 2 is based on a substrate concentration-range of about 10-fold, but one frequently finds in the literature cases where the range is even smaller so that, for example, only the lower points of curve 2*B* are available. Although a more or less accurate V_m/K_M value (intercept with the abscissa in the non-inverted plot) may be obtained in this case, it is obvious that neither V_m nor K_M can be estimated with any accuracy from such data, even though the inverted plot might tempt one to do so. Similar difficulties obtain when only results at the higher substrate concentrations, close to saturation, are available. From such data not even V_m can be estimated with certainty when substrate inhibition is involved.

Because only the v versus v/S plot shows the rates from $S \to 0$ to $S \to \infty$ on a finite graph, this plot generally demonstrates more clearly whether or not the results are sufficient for the estimation of the constants. When ample data are available, for example, over the recommended 100-fold range of substrate concentrations, the inverted plots are also the less convenient. As is seen in Fig. 2*A*, all the activities below $V_m/2$ would be found on the first small part of the curve between $1/S = 0$ and $1/S = 1$, while those above this value correspond to the range of $1/S$ between 1 and infinity. Thus, the inverted plots over-emphasize the results on one side of the curve at the cost of those on the other side. This produces a lop-sided stretching of the curve and tends to obscure deviations from linearity (see ref. 3*b*).

No titration is complete, nor the estimation of the corresponding constants accurate, if it is not carried through on both sides of the half-way point. Both these regions are of equal value in this respect. On the other hand, results obtained too far away from this point on either side are largely superfluous. From this it should be clear that the most important range of substrate concentrations for the determination of the constants are those of the order of K_M, around the value of which the curve should be symmetrical. This is the case only with the v versus v/S plot in which the half-way point is equally spaced between the two limiting values:

$$V_m \left(= \lim_{S \to \infty} v\right) \quad \text{and} \quad V_m/K_M \left(= \lim_{S \to 0} v/S\right)$$

Another largely unwarranted comment that has been made from time to time (see ref. 9) in favour of the $1/v$ versus $1/S$ plot is based on the fact that here the variables v and S are separated. With respect to estimation of the experimental error and the weighting of data, this does not constitute an argument against the v versus v/S plot when only the error in v needs to be considered, as is most often the case. In the v versus v/S plot an error in v simply displaces the experimental point along a line through the origin that

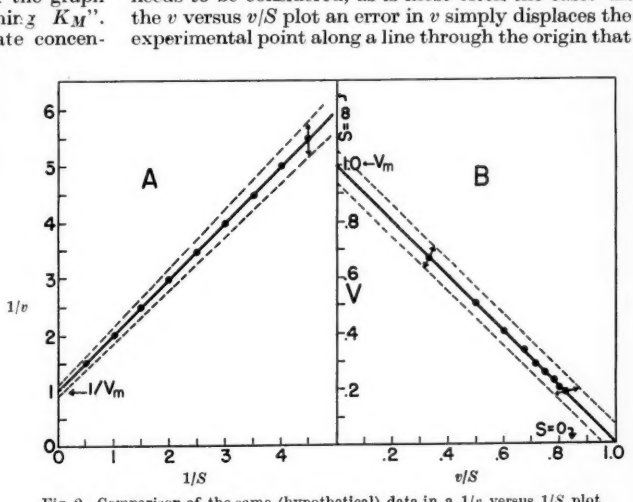

Fig. 2. Comparison of the same (hypothetical) data in a $1/v$ versus $1/S$ plot (*A*) and a v versus v/S plot (*B*), showing that (1) points that are equally distributed in the inverted plot correspond to an unfavourable distribution of rates actually measured, and (2) a limited set of results that do not allow extrapolation, that is, the points on the lower part of curve *B* are over-emphasized in the inverted plot (*A*). The arrows and the zones within the dotted lines correspond to an experimental maximum error in v of ± 5 per cent

represents a particular substrate concentration. The displacements are indicated by the arrows in Fig. 2*A* and 2*B*, where the dotted lines show the zone in which the experimental points are found when subject to a maximum error of ±5 per cent. It may be noted that in the v versus v/S plot this zone runs parallel to the theoretical curve in contrast to the inverted plot.

In view of the reasons discussed above, it would seem then that there is no real basis for the continued use of the inverted plots.

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THERE seems to be some confusion here between two things which are in reality quite distinct, namely, (*a*) the choice of the series of substrate concentrations which will give the best results, and (*b*) the best method of plotting the results obtained. These are largely independent: one is free to select the best series of concentrations without even knowing which method of plotting will be used, and when the results have been obtained one is free to plot them by either method.

With regard to the choice of substrate concentrations, we have in fact made no recommendation that the series should give equally spaced points on the reciprocal plot (namely, "equal increments of $1/S$"), as Dr. Hofstee seems to imply, nor do we recall any such recommendation by others. On the contrary, our statement that it is advantageous to have a concentration of points near the left-hand side of this plot implies approval of some such series as "equal increments of pS."

With regard to the method of plotting, both methods are of course perfectly valid, and it is our belief, based on experience of plotting results in both ways, that there is not a great deal to choose between them. This is where we differ from Dr. Hofstee, who believes that plot *B* (Fig. 2) is so greatly superior to plot *A* that there is no reason for the continued use of the latter.

The purpose of plotting is twofold: (*a*) to determine K_M and V_m, and (*b*) to check that the system obeys the Michaelis equation ('linearity', that is, of the graphs). By actual use, we find that the two methods are about equally good in both respects; the accuracy of determination of the constants from a given set of results is about the same, and it seems to us that deviations from linearity are revealed almost equally well by the two methods. We venture to think that if the reader will plot a few cases in both ways he will come to the same conclusion.

A main argument for plot *B* seems to be that a series of concentrations of the kind commonly preferred will give a more uniform distribution of points along the straight line than in the case of plot *A*. It does not follow, however, that such a uniform distribution of points will give the most accurate results; for we would point out that the position of a straight line is determined much more precisely by points near its ends than by points near its centre.

Our main reason for preferring plot *A* is that one can readily identify the different points with particular substrate concentrations, and so see what is taking place. This is not the case with plot *B*, which has no scale of substrate concentrations; the quantity which is plotted depends both on the arbitrarily fixed concentration and the resulting observed velocity, so that it is necessary to perform a division sum to discover what substrate concentration corresponds to a given point. Any error in v affects both co-ordinates, displacing the point obliquely. Also rather more calculation is involved in the actual plotting by this method. We think that many workers will continue to use plot *A*, the inverted, or as we would prefer to call it, the reciprocal plot.

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TWO-DIMENSIONAL HIGH-VOLTAGE PAPER ELECTROPHORESIS OF AMINO- AND OTHER ORGANIC ACIDS

By Dr. D. GROSS

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Amino-Acids

IT had been demonstrated before[1] that the application of high potential gradients to the electrophoresis of amino-acids leads to sharp separations after comparatively short running times. It was felt, however, that a higher degree of resolution and greater certainty of identification of the separated compounds could be attained by the adoption of a two-dimensional technique, that is, subjecting the sample to electrophoresis on the same sheet under two different *p*H conditions with consequently differing migration patterns.

A two-dimensional technique for the separation of amino-acids has been described by E. L. Durrum[2], who obtained encouraging results with mixtures of

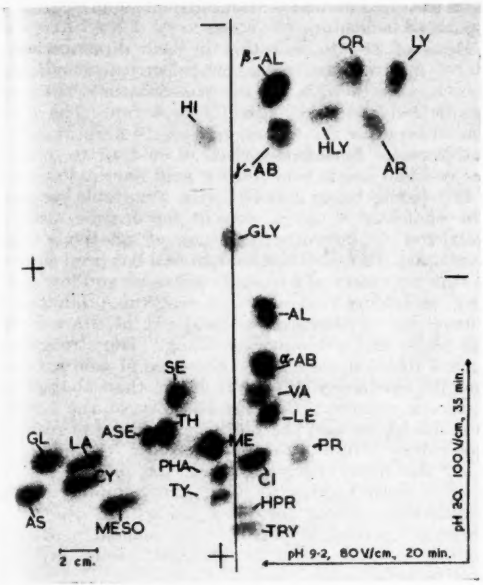

Fig. 1. Two-dimensional high-voltage electrogram of amino-acids. Conditions: first direction (bottom to top), Whatman No. 3 *MM*, 12 in. × 22½ in. 0·75 *M* formic acid, *p*H 2·0, 100 V./cm., 6·5 m.amp./cm., cooling water temperature 16° C., pressure 1·5 lb./in.², 35 min., acidified ninhydrin reagent (0·5 per cent w/v) in acetone, 10 μgm. of each amino-acid; second direction (right to left), 0·05 *M* sodium borate solution, *p*H 9·2, 80 V./cm., 9 m.amp./cm., 20 min.
Amino-acids: *α-AB*, α-amino-butyric acid; *β-AB*, β-amino-butyric acid; *γ-AB*, γ-amino-butyric acid; *AL*, α-alanine; *β-AL*, β-alanine; *AR*, arginine; *AS*, aspartic acid; *ASE*, asparagine; *CI*, citrulline; *CY*, cystine; *GL*, glutamic acid; *GLY*, glycine; *HI*, histidine; *HLY*, hydroxylysine; *HPR*, hydroxy-proline; *LA*, lanthionine; *LE*, leucine; *LY*, lysine; *ME*, methionine; *MESO*, methionine sulphone; *OR*, ornithine; *PHA*, phenyl-alanine; *PR*, proline; *SE*, serine; *TH*, threonine; *TRY*, tryptophan; *TY*, tyrosine; *VA*, valine

up to 13 amino-acids, a potential gradient of 30 V./cm. and a running time of 5 hr. Several authors[3] have since reported modifications to this technique employing relatively low voltages, but without an apparent significant increase in resolving power, expediency or speed of operation. A recently constructed apparatus of sufficient width and improved cooling efficiency made the application of high potential gradients (100 V./cm. and greater) to two-dimensional separations of amino-acids possible[4], and the technique has since been improved and usefully employed in several investigations.

The procedure found most practical is as follows: a sheet of filter paper, Whatman No. 3 *MM*, 12 in. × 22½ in., is soaked in a 0·75 *M* formic acid solution of *p*H 2·0 and blotted to remove excess moisture. The sample is applied as a streak of ½ in. width, the sheet placed in the electrophoretic apparatus (sandwich type, top and bottom cooling plates, 13 in. × 20 in., strictly controlled pressure) and connected through a 'Cellophane' membrane[5] at each end with the thick paper pads dipping into the electrolyte vessels. It is run for up to 40 min., dried for 10 min. at 90° C. and 1 hr. in a stream of cold air to remove most of the formic acid residue, trimmed to a length of 12 in., according to a developed guide-strip showing the effective length of separation, and sprayed lightly (approx. 135 per cent moisture) from an atomizer with a 0·05 *M* sodium borate solution of *p*H 9·2. It is then turned through 90°, placed carefully in the apparatus, connected by two 12 in. × 6 in. paper strips to the thick paper pads and thus to the electrode vessels, the joins being made by filling in the small gaps between sheet and strips with a slurry of cellulose powder (Whatman, standard grade) in borate solution. Simple overlapping interferes with efficient cooling and steadiness of moisture-level in the sheet. After completion of the second run, at 100 V./cm. for 20 min., the sheet is detached from the connecting strips, dried for about 15 min. at 70° C. and sprayed with a 0·5 per cent (w/v) ninhydrin solution in acetone, acidified by the addition of 3 per cent (v/v) of glacial acetic acid. It is advisable to place the sheet for spraying with borate solution on a frame provided with a grid of nylon thread and to handle moist electrograms with rubber gloves throughout, to avoid interfering finger marks.

Fig. 1 shows the pattern of 27 amino-acids and amides separated by this technique.

The position of glutamine coincides with that of methionine and, if the presence of both is suspected, it was found practicable to treat the applied sample with two drops of 30 per cent hydrogen peroxide before the first run, to convert the methionine to methionine sulphone[6] which is well separated from glutamine. The treatment only works in an acid medium. Taurine and cysteic acid can easily be separated from all the other amino-acids at *p*H 2·0 and their identification requires no confirmation by a run at a different *p*H. In this case, they would be below tryptophan, separated by a wide gap from it and from one another. The position of tryptophan at *p*H 2·0 was always found as shown and not, as J. K. Whitehead reported[3], near glycine.

Fig. 2 demonstrates the application of the technique to a commercial sample of acid-hydrolysed casein.

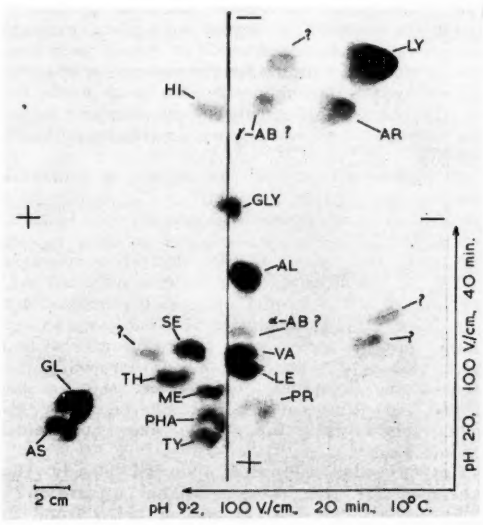

Fig. 2. Two-dimensional high-voltage electrogram of a casein hydrolysate.
Conditions: first direction (bottom to top), Whatman No. 3 *MM*, 12 in. × 22½ in., 0·75 *M* formic acid, *p*H 2·0, 100 V./cm., 6 m.amp./cm., cooling water temperature 10° C., pressure 1·5 lb./in.², 40 min., ninhydrin reagent as in Fig. 1, 250 μgm. of hydrolysate; second direction (right to left), 0·05 *M* sodium borate solution, *p*H 9·2, 100 V./cm., 9·5 m.amp./cm., 20 min.
Amino-acids: as in Fig. 1

Most of the common amino-acids can be identified by their positions; the appearance of some spots in unusual positions may be due to the presence of peptides or other ninhydrin-reactive compounds. No attempt has been made to identify the compounds against which question marks are placed. The partial overlapping of valine and leucine (or the leucines) is due to their relatively high concentrations. The slight difference in migration distances in the second direction is due to a lower temperature and moisture content of the sheet. Under standardized conditions the degree of reproducibility of the spot patterns is usually high. The time for the separation of less complex mixtures can be reduced in many cases to about one half.

Other Organic Acids

As a result of a systematic study of the migration-rates of non-volatile organic acids under varying conditions of *p*H, ionic strength and composition of background electrolyte[7], it was found possible to improve greatly the resolution of complex mixtures by choosing three well-tested buffer or electrolyte solutions of *p*H 2·0, 4·0 and 8·9 with advantageous differences in rate and sequence of migration.

With the availability of an improved electrophoretic apparatus of sufficiently large dimensions[4], the feasibility of a two-dimensional technique became apparent and was successfully tried. The two-dimensional technique, as in paper chromatography, allows the best use of the resolving power inherent in the electrolyte systems chosen, particularly in conjunction with the application of a high-voltage technique ensuring clean separations with a minimum of diffusion and liquid flow effects. This high degree of resolution is particularly desirable when dealing with a multitude of organic acids as potentially present in complex biological and plant extracts. The previously described[8] 0·75 *M* formic acid solution, found most suitable for the separation of strong and moderately strong acids from weak acids, has the disadvantage of largely suppressing the ionization of the weak acids, with attendant loss of mobility.

At higher *p*H values the degree of ionization increases appreciably and with it the mobilities of the weak acids, which makes separation and identification of the individual acids based on their varying migration-rates feasible. Two electrolyte solutions, namely, a 0·5 *M* acetic acid solution adjusted with pyridine to *p*H 4·0, and an approximately 0·1 *M* ammonium carbonate solution of *p*H 8·9, were found to offer suitable variations in migration-rates without the formation of multiple spots resulting from partial dissociation. Ammonium carbonate solution had proved its usefulness in the electrophoresis of inorganic acids, volatile fatty acids[9] and some non-volatile organic acids[10].

The procedure adopted followed closely that developed for the two-dimensional separation of amino-acids. The first run was at *p*H 8·9 and the second run at *p*H 4·0 or 2·0. The sheet was soaked in the electrolyte and blotted before streaking the sample over a width of 2·5 cm., run and dried for 15 min. at 90° C. Before the second run, the electrolyte was lightly sprayed from an atomizer on both sides of the paper sheet to a sufficient degree of moistness (about 135 per cent on dry paper) to ensure electrical conductance without displacing the partially separated acids.

Runs of 25-min. duration in each direction were found appropriate under the electrical conditions chosen, though with less complex mixtures the time can be reduced to 15 min. for each run. The sheet was dried after the second run for 10 min. at 85° C. and kept for 1 hr. in a stream of cold air to remove the residual acetic acid (formic acid required at least 3 hr.) before being sprayed with a suitable reagent. The choice of a useful reagent for organic acids is restricted to indicator solutions of relatively high sensitivity (provided the background has been cleared of residual traces of free acids or bases) and low stability of colours and chemical reagents producing a colour due to the presence and *p*H of the acid on the paper and subsequent heating. The strength of such a reaction varies with the type of acid present, and its sensitivity is usually lower than that of an indicator reagent; but the stability of the colours is much higher and the presence of traces of residual electrolyte is not so critical.

Of the many reagents tried, the aniline–glucose reagent with heating at 115° C. was found to give satisfactory results for most acids, and the ferric chloride – potassium ferricyanide reagent[11] without heating proved useful for syringic, lactic, glycollic and tartaric acid.

A 0·025 per cent solution of bromocresol purple in ethanol–water (75 : 25) provided a useful and sensitive indicator reagent which could also be applied prior to spraying with the aniline–glucose reagent, the latter providing a permanent record of brown spots on a near-white background. Picric acid, a coloured compound, was used as a marker to indicate the progress of migration.

An example of a separation of twenty organic acids is illustrated by the electrogram in Fig. 3.

Thanks are due to Mr. R. W. Butters for valuable technical assistance, Mr. D. G. Harrison for skilful

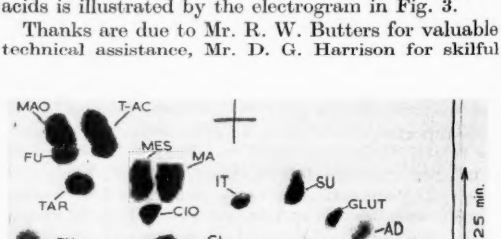

Fig. 3. Two-dimensional separation of organic acids by high-voltage electrophoresis.

Conditions: first direction (bottom to top), Whatman No. 3 *MM*, 12 in. × 22½ in., approx. 0·1 *M* ammonium carbonate solution (7·9 gm./l.), *p*H 8·9, 70 V./cm., 8·2 m.amp./cm., cooling water temperature 12° C., 1·5 lb./in.², 25 min.; second direction (right to left), 0·5 *M* acetic acid adjusted with pyridine to *p*H 4·0, 100 V./cm., 10 m.amp./cm., 25 min.; 50 μgm. of each acid. Spraying reagent: glucose-aniline (approx. 2 per cent of each) in ethanol–water (2 : 8).

Organic acids: *AD*, adipic; *AS*, ascorbic; *CI*, citric; *CIO*, citraconic; *FU*, fumaric; *GLUT*, glutaric; *GLYC*, glycollic; *IT*, itaconic; *LA*, lactic; *LAE*, lævulinic; *MA*, malic; *MAO*, malonic; *MEC*, meconic; *MES*, mesaconic; *PI*, picric; *PLC*, pyrrolidone-carboxylic; *PY*, pyruvic; *SU*, succinic; *T-AC* *trans*-aconitic; *TAR*, tartaric

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ACTION OF MANGANESE DIOXIDE ON SIMPLE CARBOHYDRATES

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UNDER mild conditions, manganese dioxide (MnO_2) selectively oxidizes $\alpha\beta$-unsaturated alcohols to the corresponding carbonyl compounds[1]; N-alkyl and N,N-dialkylanilines are also attacked, yielding amides[2]. Elevation of the temperature (70–120°) results in a reduced selectivity of oxidizing action and a variety of reactions have been observed[1,3], some of which were carried out in aqueous media. We now report a preliminary qualitative survey of the action of manganese dioxide on simple carbohydrates.

Initially the activity of manganese dioxide preparations obtained by the following methods were compared: (1) alkali-permanganate[4], (2) acid-permanganate[5], (3) decomposition[6] of manganese oxalate at 250°, (4) decomposition[6] of manganese carbonate at 250°, (5) preparations (1)–(4) after nitric acid treatment[6]. The alkali-permanganate product gave an aqueous extract *p*H *c.* 10 even after multiple treatments with water, but the remainder gave neutral extracts. Three reaction conditions were employed in which a solution of the carbohydrate (50 mgm.) in water (1·5 ml.) was vigorously shaken with manganese dioxide: (*A*) 50 mgm. at 50° for 1 hr., (*B*) 50 mgm. at 95–100° for 1 hr., (*C*) 150 mgm. at 95–100° for 1 hr. These ratios of oxidant to substrate are considerably lower than those often used with other types of compound[1]. The filtered solutions were examined by (1) paper chromatography (downward irrigation with the organic phase of a butanol/ethanol/water (4 : 1 : 5) solvent system); (2) paper ionophoresis (enclosed strip technique[7]) with a borate buffer[8] *p*H 10 and an acetate buffer *p*H 5 and detection with aniline hydrogen phthalate[9] and alkaline silver nitrate[10]. Identification of products must be considered tentative, although the application of chromatography and ionophoresis in conjunction permits a more certain identification[11]. The chain-length of each acid formed in the oxidations was determined by ionophoresis in acetate buffer, and mobilities expressed as M_{GA} values (GA = gluconic acid) give the sequence: gluconic acid 1·00, arabonic acid 1·09, erythronic acid 1·27, glyceric acid 1·54 and glycollic acid 1·77.

Slight differences were observed in the effect of the various manganese dioxide preparations on glucose and fructose. The subsequent results were obtained with manganese dioxide prepared from the carbonate since it is the easiest and cheapest to prepare.

Aldoses and related compounds. From Table 1 it may be seen that the hexoses yielded pentoses together with acidic products. The hexose → pentose conversion is not a normal oxidation pathway of sugars, although it can be effected by glycol cleavage reagents[12], and amino-sugars may be degraded to pentoses by ninhydrin[13]. Both hexose and pentose were obtained *inter alia* from the heptose. The susceptibility of erythrose to oxidation was quite striking; it reacted completely under conditions *B* and *C* and hence did not appear as an oxidation product of the higher sugars other than of pentoses under condition *A*.

When galactose was treated under condition *A* with manganese dioxide, obtained from the alkali-permanganate reaction, in addition to the products shown in Table 1 traces of erythrose could also be detected, and under condition *B* epimerization occurred yielding talose and tagatose. This observation is not surprising in view of the alkalinity of the reaction solution.

Table 1. Oxidation of Aldoses and Derivatives with Manganese Dioxide

Aldose	Reaction condition	Products*
Galactose†	*A*	Galactose, trace lyxose
	B	Galactose, lyxose, traces of 5C and 4C acids
	C	Galactose, lyxose 6C, 5C, 4C and 3C acids
D-*Glycero*-D-*galacto*-heptose	*A*	Heptose, trace mannose
	B, *C*	Heptose, mannose, traces of arabinose and 6C acid
Ribose‡	*A*	Ribose, trace erythrose, 4C acid
	B, *C*	Ribose, arabinose, 5C, 4C and 3C acids
Erythrose	*A*	Erythrose, 3C acid
	B, *C*	4C, 3C and traces of other acids (erythrose completely oxidized)
2-Deoxy-D-'galactose' 2-Acetamido-2-deoxy-D-glucose	*A*, *B*, *C*	Traces of unidentified products
3-O-methyl glucose	*A*	3-O-methyl-glucose, trace 2-O-methyl-arabinose, 6C acid
	B, *C*	3-O-methyl-glucose, 2-O-methyl-arabinose, 6C acid
Rhamnose	*A*	Rhamnose, 5-deoxy-arabinose, 5C acid
	B, *C*	Rhamnose, 5-deoxy-arabinose, 5C, 4C, 3C acids
2-Amino-2-deoxy-D-glucose hydrochloride	*A*, *B*, *C*	Complex mixture of products

* Traces of unidentified compounds were detected in several cases and manganese ions were invariably present. This comment is also applicable to the results in Tables 2 and 3.
† Parallel results with glucose, mannose and other hexoses.
‡ Parallel results with xylose and arabinose.

Table 2. Action of Manganese Dioxide on some Reducing Disaccharides

Disaccharide	Linkage	Reaction condition	Products*
Sophorose	β 1 → 2	*A, B, C*	Insignificant reaction
Laminaribiose (*MG* 0·66)	β 1 → 3	*A*	Laminaribiose, trace *G-A* (*MG* 0·33), trace acid P (*MGA* 0·73)
		B, C	Laminaribiose, *G-A*, P, glucose, arabinose
Maltose (*MG* 0·30)	α 1 → 4	*A*	Maltose, trace *G-A* (*MG* 0·56), trace *G-T* (*MGA* 0·76)
		B, C	Maltose, *G-A*, *G-T*, glucose
Cellobiose (*MG* 0·26)	β 1 → 4	*A*	Cellobiose, trace *G-A* (*MG* 0·57), trace *G-T* (*MGA* 0·77)
		B, C	Cellobiose, *G-A*, *G-T*, glucose, arabinose, *Q* (*MG* 0·30)
Melibiose (*MG* 0·74)	α 1 → 6	*A*	Melibiose, trace *Gal-A* (*MG* 0·87), trace *R* (*MG* 0·42)
		B, C	*Gal-A*, *R*, trace acid *S* (*MGA* 0·94), trace galactose and lyxose

* *G-A* indicates glucosyl-arabinose. *G-T* indicates glucosyl-tetronic acid. *Gal-A* indicates galactosyl-arabinose. *MG* value is the ionophoretic mobility in borate buffer with respect to that of glucose (ref. 11), *RG* value is the paper chromatographic mobility with respect to that of glucose.

Enhanced resistance to oxidation is conferred by the absence of a C_2-hydroxyl group (2-deoxy-D-'galactose') and substitution at C_2 (2-acetamido-2-deoxy-D-glucose). The results with 3-O-methyl-D-glucose and rhamnose indicate that manganese dioxide oxidations might have some value for the synthesis of, for example, O-methyl- and deoxy-pentoses. Although a chromatographic separation of the product would be necessary, application of this technique is also frequently essential in the more classical synthetic methods.

Reducing disaccharides. In examining the behaviour of disaccharides with manganese dioxide the analytical methods noted above were supplemented as follows. The acidic products were separated from the neutral components by absorption[14] on 'Deacidite *FF* 530' ($CO_3^{=}$ form) followed by elution with ammonium carbonate. The neutral and acidic products were then hydrolysed separately with 2 *N*-hydrochloric acid at 95–100° for 3 hr., and after neutralization with methyl di-*n*-octylamine[15], the hydrolysates were examined by chromatography and ionophoresis.

From Table 2 it is seen that the disaccharides yielded less complex mixtures on oxidation than did the aldoses. The 1 → 2 linked disaccharide (sophorose) was largely unaffected by manganese dioxide, whereas the 1 → 3, 1 → 4 and 1 → 6 linked disaccharides were each degraded to give mainly a glucosyl–pentose together with a small amount of apparently a glycosyl–glyconic acid. Small amounts of hexose and pentose were also formed, but no 6-carbon or smaller acids. As might be expected from the aldose oxidations, glucosyl–tetroses did not accumulate on oxidation of the 1 → 4 and 1 → 6 linked disaccharides. The use of a large excess of oxidant did not completely convert maltose into its oxidation products. These results suggest that manganese dioxide oxidation might provide a convenient method for converting suitable hexosyl hexoses into hexosyl-pentoses. (Glycol cleavage reagents might also be used for this purpose[12].) Although a column chromatographic separation would be necessary to isolate the required product, it should be noted that, with the 1 → 3 and 1 → 4 linked glucosyl–glucose oxidation mixtures, the markedly different M_G values of the glucosyl–glucose and the glucosyl–pentose create an ideal situation for the application of borate–charcoal–'Celite' chromatography[16]. Further, the sensitivity of the 1 → 3 linked disaccharide (laminaribiose) towards lime-water permits[17] complete destruction of unoxidized disaccharide, leaving the 1 → 2 linked glucosyl–pentose unaffected.

Ketoses and miscellaneous compounds. From Table 3 it is seen that fructose variously yielded tetrose or underwent epimerization in addition to the formation of acids. Inosose, sodium gluconate and glucurone were readily oxidized, but most of the products of these reactions have not been identified and they are being actively investigated. The conversion of hexitols to hexoses and pentoses is of interest. The symmetrical hexitols (galactitol and mannitol) gave a single hexose and pentose, whereas glucitol gave two hexoses and two pentoses, indicating attack at both ends of the carbon chain.

It is clear that a wide range of carbohydrates is attacked by manganese dioxide and that a variety of oxidation pathways is operative. Some of the reactions may be of potential value for the preparation of otherwise inaccessible carbohydrates.

A number of commercially available oxides were also examined for action on galactose and fructose under condition *A*.

Zinc oxide (ZnO), cadmium oxide (CdO), nickel oxide (Ni_2O_3) and black and grey cobalt oxide (Co_2O_3—Co_3O_4) caused slight epimerization.

Zirconium dioxide (ZrO_2) and lead oxides (PbO_2, PbO and Pb_3O_4) showed weak manganese dioxide-type properties (namely, galactose → lyxose), but PbO and Pb_3O_4 effected slight epimerization. There was negligible effect on fructose. Under condition *B* almost complete destructive oxidation of the substrates occurred with the lead oxides.

The following oxides displayed no oxidative action: HgO (red and yellow), Hg_2O, UO_3, Cu_2O, CuO, Bi_2O_3, SnO, SnO_2, MoO_3, Nb_2O_5, Y_2O_3, Sb_2O_3, Fe_2O_3, Fe_3O_4, GeO_2, V_2O_5, Cr_2O_3, TiO_2.

Table 3. Oxidation of Carbonyl-containing and Other Carbohydrates

Compound	Oxidation condition	Products
Fructose*	*A*	Fructose, tetrose, 5C acid
	B, C	Fructose, glucose, mannose, 6C, 5C, 4C and 3C acids
Inosose	*A, B, C*	Inosose, mixture of acids and reducing acids
Sodium glucuronate†	*A*	No significant effect
	B, C	Glucuronic acid, trace of 4C, 3C acids, dicarboxylic acid and other unidentified components
Sodium gluconate‡	*A*	Gluconic acid, reducing acid, trace arabinose
	B, C	Gluconic acid, reducing acid, arabinose, unidentified components
Potassium glucarate	*A, B, C*	Glucaric acid, traces of reducing and acidic products
Galactitol§	*A*	Galactitol, trace galactose
	B, C	Galactitol, galactose, lyxose
Glucitol	*A*	Glucitol, trace glucose and gulose
	B, C	Glucitol, glucose, gulose, xylose and arabinose
Methyl α-D-glucopyranoside	*A, B, C*	Traces of unidentified products
Sucrose	*A*	Sucrose, trace of reducing component *S* (*MG* 0·41)
	B, C	Sucrose, *S*, glucose, fructose and acid T (*MGA* 0·73)

Parallel results with (*) sorbose, (†) glucurone, (‡) glucono-δ-lactone, (§) mannitol.

We thank Miss B. Parkinson for experimental assistance.

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CROSS-LINKING OF CELLULOSE ACETATE BY IONIZING RADIATION

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RECENT studies on the polymerization induced by radiation of allyl esters have revealed that the conversion-rate is markedly increased in the presence of polyvinyl chloride, while at the same time the polyvinyl chloride acquires a high density of cross-linking[1]. Polyvinyl chloride belongs to the class of polymers which undergo cross-linking by radiation[2], so that the presence of the allyl ester serves to enhance the action of the radiation but no alteration of mode is involved. It is known that such alterations can occur; for example, in the presence of oxygen, many polymers which are normally cross-linked by radiation may become degraded[3]. An artificial means of rendering a change in the reverse direction constitutes a worth-while objective, as the benefits of cross-linking could then be extended to a large class of polymers such as poly*iso*butylene and cellulose derivatives, but such means have not hitherto been described.

The enhancement of the density of cross-linking in polyvinyl chloride by the presence of the allyl ester was of such magnitude that it seemed feasible to cross-link by radiation, in this way, those polymers the normal response of which to radiation is degradation. Experiments with poly*iso*butylene and polymethyl methacrylate have given ostensible evidence of cross-linking while with secondary cellulose acetate the results leave no shadow of doubt that heavy cross-linking can be achieved. This polymer, like polyvinyl chloride, is normally plasticized to facilitate processing and advantage could thus be taken of the plasticizing action of the allyl ester.

The most obvious effect of replacement of normal plasticizers in cellulose acetate by a convertible plasticizer such as triallyl citrate was that irradiation caused a pronounced increase in tensile strength. This is shown in Fig. 1, where the tensile strength at room temperature of a composition initially containing 32 per cent triallyl citrate is given as a function of the radiation dose, using 2-MeV. electrons from the scanned beam of a Van de Graaff accelerator. As shown in the accompanying curve, normally plasticized cellulose acetate exhibited no change in tensile strength over the dose-range studied, despite the radiation damage which was betrayed by darkening, bubbling and reduction in ultimate elongation.

Elevation of the tensile strength at room temperature provided evidence for the polymerization of the triallyl citrate but not, in itself, of cross-linking of the cellulose acetate. The accepted criteria for cross-linking are non-fusibility, non-solubility and reversible high elasticity. Measurements of fusibility were inappropriate in the present instance because of the high melting point of secondary cellulose acetate (*c.* 300°) (ref. 4). Measurements of rubber-like behaviour were similarly thwarted by the inherent stiffness of cellulose acetate chains, and only solubility and swelling criteria can be employed. As is well known, secondary cellulose acetate can be dissolved rapidly in acetone. By contrast, irradiated solid solutions of secondary cellulose acetate in triallyl citrate were highly resistant to acetone. This is shown in Fig. 2, where for a composition containing 32 per cent triallyl citrate, the gel fraction (24 hr. Soxhlet extraction with acetone) and the swelling index (24 hr. in acetone at room temperature) are given as a function of dose. Even a dose as low as 10 Mrads has sufficed to confer a considerable degree of solvent resistance, matching that of similarly cross-linked polyvinyl chloride[1].

For the purpose of exploring the mechanism whereby cellulose acetate has been rendered insoluble, it was desirable to measure the rate of polymerization induced by radiation of the triallyl citrate. Such measurements in the solid state pose a number of problems. Physical methods are generally superior, and density, spectroscopic and relaxation methods have been used[5]. In the present instance, the rate of conversion of the ester has been followed using a Beckman *DK*2 spectrophotometer. Solutions of triallyl citrate in acetone were used to construct a calibration curve relating the absorbance of the 1·625μ allylic absorption band with the allyl concentration and this curve led, with appropriate corrections, to values for residual allyl concentration

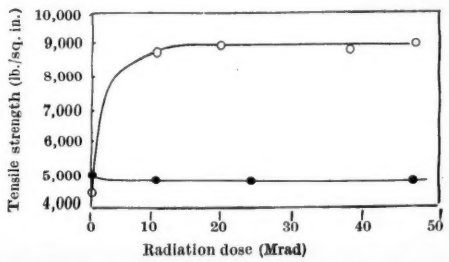

Fig. 1. Tensile strength of irradiated cellulose acetate. ●, Normally plasticized; ○, plasticized with 32 per cent triallyl citrate

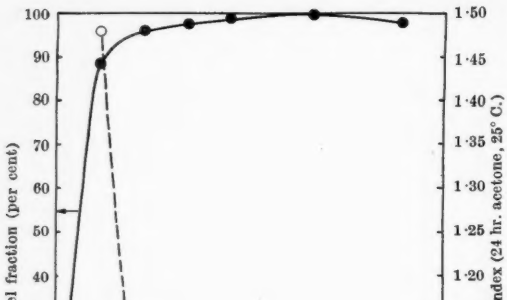

Fig. 2. Solubility and swelling of irradiated cellulose acetate. ●, Gel fraction (24 hr. Soxhlet, acetone); ○, swelling index (24 hr. acetone, 25° C.)

in irradiated solid solutions of cellulose acetate in triallyl citrate. The results are shown in Fig. 3, whence, allowing for initial scatter, it is apparent that the disappearance of allyl is essentially proportional to dose up to at least 50 per cent conversion and that the slope is essentially independent of concentration.

The average initial allyl disappearance-rate was 0·202 micromole per gram per megarad, corresponding to G (-allyl) = 200. Corresponding figures for the electron-induced homopolymerization-rate are not available, but in view of the relatively small intensity dependence of allyl polymerization, comparison may be made with the rate of homopolymerization induced by γ-rays, which at an intensity of 11,700 rads/min. gives G (-allyl) = 103 (Wycherley, V., unpublished work). It follows that while some enhancement of conversion-rate in the presence of cellulose acetate has occurred, the effect is small.

As an initial hypothesis, it may be considered that the components of the solid solution of cellulose acetate in triallyl citrate respond independently to radiation in their characteristic fashion. The hexafunctional monomer when irradiated alone has yielded gel at approximately 10 per cent conversion (Wycherley, V., unpublished work). The rate of radiolysis of cellulose acetate has not been reported, the closest approximation being the fracture-rate for irradiated cellulose, which has been given as G (fracture) = 11 (ref. 6). If these processes are superimposed, it follows that the cellulose acetate component will be progressively rendered insoluble by irradiation of the mixture if it becomes attached to the allyl network at a rate exceeding that of radiolysis. The mode of attachment is simply that of initiation of an allyl chain, and it remains to consider semi-quantitatively the probable rate of initiation by polymeric cellulose acetate radicals relative to that of scission.

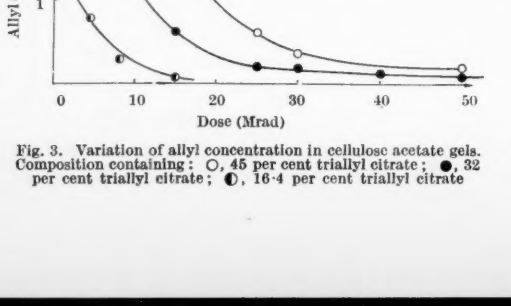

Fig. 3. Variation of allyl concentration in cellulose acetate gels. Composition containing: ○, 45 per cent triallyl citrate; ●, 32 per cent triallyl citrate; ◐, 16·4 per cent triallyl citrate

The ratio of frequency of chain transfer to frequency of propagation during normal polymerization of allyl esters lies in the range 10–20 (ref. 7). Taking the mean for triallyl citrate leads to G (chain initiation) $\simeq 13$. Since the condition for cross-linking is that the probability of forming cross-linking units should be at least half the probability of dislinking, that is, $p_0/q_0 < 2$, the minimum G-value for junction point formation on the cellulose acetate in order for gel to accumulate is 5·5. While it is entirely reasonable that the G value for radical formation produced by radiation in cellulose acetate might equal 5·5, such a rate of initiation could only lead to very slow aggregation of the gel. In point of fact, gel aggregation is rapid (see Fig. 2), which is more consistent with a value of q_0 exceeding p_0. While G values for initiation by cellulose acetate as high as 10–15 cannot be ruled out, on present limited data, such primary initiation-rates seem abnormally high and it is more likely that a supplementary contribution is made by a process of effective chain-transfer, whereby a growing allyl chain abstracts a hydrogen atom from the cellulose acetate molecule rather than from an allyl monomer molecule, leaving the resultant macroradical to re-initiate a further allyl chain and serve as a junction point with the network. In view of the similarity in the triallyl citrate conversion-rate in the presence or absence of cellulose acetate, there is no need, however, to postulate reactivation of resonance-stabilized allylic radicals, as was found necessary in the case of polyvinyl chloride[1].

If this picture is correct, it may be concluded that cross-linked cellulose acetate arises as a consequence of irradiation in the presence of a network-forming monomer, which has a short propagation chain-length and serves as an efficient trap for polymeric radicals whether produced directly by radiation or indirectly by hydrogen abstraction.

This work has been carried out in collaboration with B.X. Plastics, Ltd., and we wish to thank Dr. R. R. Smith and Dr. M. Pettit for discussion, and the Chairman of B.X. Plastics, Ltd., and the Chairman of Tube Investments, Ltd., for permission to publish this work.

[1] Pinner, S. H., *Nature*, **183**, 1108 (1959).
[2] Chapiro, A., *Indust. des Plastique Modernes*, **8**, No. 1, 41 (1957); *J. Chim. Phys.*, **53**, 895 (1956).
[3] Alexander, P., and Charlesby, A., *J. Polymer Sci.*, **23**, 355 (1957).
[4] Malm, C. J., *et al.*, *Indust. Eng. Chem.*, **43**, 684 (1951).
[5] Pinner, S. H., and Wycherley, V., paper submitted to Wiesbaden Conference on High Polymers.
[6] Charlesby, A., *J. Polymer Sci.*, **15**, 263 (1955).
[7] Bartlett, P. D., and Altschul, R., *J. Amer. Chem. Soc.*, **67**, 816 (1945).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, November 30

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 2.30 p.m.—Anniversary Meeting.

INSTITUTE OF METAL FINISHING (in the Canterbury Room of the Charing Cross Hotel, London, W.C.2), at 2.45 p.m.—Mr. A. A. B. Harvey: "The Role of the Scientific Society" (Presidential Address).

UNIVERSITY COLLEGE (in the Physiology Theatre, Gower Street, London, W.C.1), at 5 p.m.—Prof. E. P. Kennedy (University of Chicago): "The Biosynthesis of Complex Lipids".* (First of two lectures in Biochemistry. Further lecture on December 7.)

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Dr. L. Pearce Williams: "Faraday Through His Manuscripts".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Prof. C. von Fürer-Haimendorf: "Sherpas of Eastern Nepal".

Tuesday, December 1

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Prof. R. E. D. Bishop: "Vibration Problems in Engineering".*

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND ELECTRONICS SECTIONS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. L. Essen, Mr. J. V. L. Parry and Mr. J. McA. Steele: "Frequency Variations of Quartz Oscillators and the Earth's Rotation in Terms of the N.P.L. Cæsium Standard".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. H. K. Porter: "Physiology has No Frontiers" (Inaugural Lecture).

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. L. Gowans: "The Lymphocyte".* (Twelfth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation. Further lectures on December 3, 8, 10.)

PLASTICS INSTITUTE (at the Wellcome Building, 183–193 Euston Road, London, N.W.1), at 6.30 p.m.—Mr. M. R. E. Ashenden: "Plastics and the Law".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Dr. I. M. Hall: "Transonic Flow Over Swept Wings".

Wednesday, December 2

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 p.m.—Mr. E. M. L. Beale: "Confidence Regions in Non-Linear Estimation".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. J. Marechal and Mr. P. de Radzitsky: "Potentialities of Urea in Dewaxing Middle and Heavy Distillates".

INSTITUTE OF INFORMATION SCIENTISTS (at the Berners Hotel, 10 Berners Street, London, W.1), at 6 p.m.—Discussion on "Languages in Information Work—To What Extent is Competence in a Foreign Language an Essential Qualification for an Information Scientist?".

Wednesday, December 2—Thursday, December 3

IRON AND STEEL INSTITUTE (in the Great Hall, Caxton Hall, Caxton Street, London, S.W.1, and the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 9.30 a.m. daily—Autumn General Meeting.

Thursday, December 3

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. P. R. Bell: "The Origin of Indian Corn".*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. F. H. C. Edgcombe and Prof. R. G. W. Norrish, F.R.S.: "A Study of the Mechanism of Photochemical Electron Transfer Processes in Solution"; Mr. I. M. Dawson and Mr. E. A. C. Follett: "An Electron Microscope Study of Synthetic Graphite".

INSTITUTE OF MARINE ENGINEERS (joint meeting with the INSTITUTE OF NAVAL ARCHITECTS, in the Weir Hall, 10 Upper Belgrave Street, London, S.W.1), at 4.45 p.m.—Prof. G. Aertssen: "New Sea Trials on the Sandblasted *Lubumbashi*".

UNIVERSITY OF LONDON (at the London School of Economics and Political Science, Houghton Street, London, W.C.2), at 5 p.m.—Dr. E. R. Leach: "Rethinking Anthropology"* (Malinowski Memorial Lecture).

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—Mrs. Mildred Valley Thornton: "Indians of British Columbia".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. C. B. B. Wood and Mr. I. J. Shelley: "The Transmission of News Film over the Trans-Atlantic Cable".

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (joint meeting with the SOCIETY FOR APPLIED BACTERIOLOGY, at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 6.15 p.m.—Dr. F. Brown: "Infective Ribonucleic Acid from the Virus of Foot-and-Mouth Disease".

ROYAL PHOTOGRAPHIC SOCIETY, MEDICAL GROUP (at 16 Princes Gate, London, S.W.7), at 7 p.m.—Sir Stanford Cade, K.B.E., C.B., F.R.C.S.: "What I Want from a Medical Photograph".

Friday, December 4

INSTITUTION OF ELECTRICAL ENGINEERS, MEDICAL ELECTRONICS DISCUSSION GROUP (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Nuclear Magnetic Resonance", opened by Dr. N. Sheppard and Dr. R. E. Richards.

SOCIETY OF DYERS AND COLOURISTS (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 6 p.m.—Mr. R. C. Oakley: "Dyeing of Ribbons"; Mr. R. Woods: "Dyeing of Carpet Yarns".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. H. A. Thomas: "Electronic 'Brains'".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN PHYSICS—The Registrar, The University, Manchester 13 (November 30).

CHAIR OF CHEMICAL ENGINEERING—The Registrar, University College, Singleton Park, Swansea (November 30).

ASSISTANT LECTURER IN PHYSIOLOGY—The Registrar, The University, Sheffield (December 5).

LECTURER IN PHYSIOLOGICAL PSYCHOLOGY—The Registrar (Room 22, O.R.B.), The University, Reading (December 9).

LECTURER IN SOCIAL ANTHROPOLOGY, and a LECTURER IN RURAL SOCIOLOGY in the FACULTY OF ECONOMIC AND SOCIAL SCIENCES—The Registrar, The University, Manchester 13 (December 12).

ORGANIC CHEMIST, Scientific Officer or Senior Scientific Officer grade, IN THE DEPARTMENT OF HOP RESEARCH, for research problems related to the hop plant—The Registrar, Wye College, near Ashford, Kent (December 12).

ASSISTANT LECTURER OR LECTURER IN APPLIED MATHEMATICS—The Registrar, The University, Sheffield (December 19).

ASSISTANT LECTURER OR LECTURER IN THE DEPARTMENT OF ZOOLOGY—The Registrar, The University, Liverpool (December 19).

LECTURER/SENIOR LECTURER (with an interest in, and qualified for teaching and research in any of the main branches of psychology) IN PSYCHOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, December 19).

LECTURER IN FOREST PATHOLOGY at the Waite Agricultural Research Institute—The Registrar, University of Adelaide, Adelaide, South Australia (December 21).

SENIOR LECTURER IN APPLIED ANATOMY at the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, December 21).

SENIOR LECTURER OR LECTURER IN PSYCHOLOGY at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 22).

SENIOR PRINCIPAL SCIENTIFIC OFFICER (honours graduate in science with appropriate research experience) with the Hill Farming Research Organization, to act as Deputy to the Director of the organization and, in particular, to be responsible to him for the development and co-ordination of the programme of research carried out by the Organization's scientific departments—The Secretary, Hill Farming Research Organization, 48 Palmerston Place, Edinburgh 12 (December 31).

ASSISTANT LECTURER OR LECTURER IN PHYSICS—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (January 2).

READER IN MEDICAL MICROBIOLOGY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (January 15).

LECTURER OR ASSISTANT LECTURER in PSYCHOLOGY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (Janaury 16).

SENIOR LECTURER and a LECTURER IN MATHEMATICS at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (January 29).

ADMINISTRATIVE ASSISTANT (male science graduate (preferably physicist) not more than 35 years old, and interested in space research) with the Secretariat of the British National Committee on Space Research—The Assistant Secretary, Royal Society, Burlington House, Piccadilly, London, W.1.

AREA CHEMIST (with high academic and/or professional qualifications, good general experience of modern analytical methods, and preferably well versed in the technology of lubricating oils and greases—The Director of Research (British Railways), British Transport Commission, 222 Marylebone Road, London, N.W.1.

BIOLOGIST (with a good honours degree in zoology and/or botany, preferably a knowledge of both subjects and some postgraduate experience) with the Fauna Research Unit, Kenya, to carry out research on Kenya fauna to provide data for the building up of life tables; details of feeding habits, enemies, predators and diseases—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. No. BCD.61/7/09/C1.

BOTANIST (PLANT PATHOLOGIST) (with a good honours degree in botany or agricultural botany, and at least two years postgraduate experience in plant pathology) in the Department of Agriculture, Uganda, to diagnose the cause of crop diseases and recommend control measures, and to assist with the breeding of disease-resistant crops—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. No. BCD.63/9/02/C1.

ENTOMOLOGIST, Scientific or Senior Scientific Officer (with a good honours degree in zoology with postgraduate training or experience in entomology) at the West African Cocoa Research Institute, Ghana, for research on insect pests of cocoa—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. No. BCD.197/200/02/C1.

GROSS ANATOMIST (with research interests preferably, but not exclusively, either in electron microscopy or optical (especially interference) microscopy)—The Chairman, Department of Anatomy, Emory University, Atlanta 22, Georgia, U.S.A.

IRRIGATION RESEARCH OFFICER (with a university degree in agriculture or a science degree with agricultural experience, and training or experience in soil physics or irrigation) in Swaziland, to carry out irrigation experiments on sugar cane, citrus and other crops, and to take charge of a new experimental station—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/76/014/T.

LABORATORY TECHNICIAN (able to work with the minimum of supervision, a sound knowledge of chemistry, and some experience of biological techniques)—The Administrator, Department of Biochemistry, The University, South Parks Road, Oxford.

LECTURER (well qualified physicist, and preferably with experience of the application of physics in industry) IN PHYSICS—The Registrar, Bradford Institute of Technology, Bradford 7.

MASTER to teach Chemistry, principally in the Sixth Form, up to scholarship standard, with some Middle School work—The Headmaster, University College School, Frognal, Hampstead, London, N.W.3.

PLASTICS CHEMIST OR PLASTICS TECHNOLOGIST IN THE DEPARTMENT OF MATERIALS, to assist in the organization and supervision of the teaching laboratories and to participate in the research activities of the plastics group—The Registrar, The College of Aeronautics, Cranfield, Bletchley, Bucks.

TECHNICIAN OR SENIOR TECHNICIAN IN THE ZOOLOGY DEPARTMENT—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey.

TECHNICIAN (preferably with previous experience in bacteriological work), to work in the Virus Research Laboratory at Lodge Moor Hospital—Prof. C. H. Stuart-Harris, The Royal Hospital, Sheffield 1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Hannah Dairy Research Institute. Report for the Three Years ended 31st March, 1959. Pp. 52+12 plates. (Kirkhill, Ayr: Hannah Dairy Research Institute, 1959.) [1410

The Needs of Youth in Stevenage: a Report to the Calouste Gulbenkian Foundation. Pp. 47. (London: Calouste Gulbenkian Foundation, United Kingdom and British Commonwealth Branch, 1959.) 3*s*. [1410

Department of Scientific and Industrial Research: Road Research Laboratory. Road Research Technical Paper No. 45: Investigation of the Performance of Pneumatic-tyred Rollers in the Compaction of Soil. By W. A. Lewis. Pp. iv+44+4 plates. (London: H.M. Stationery Office, 1959.) 3*s*. 6*d*. net. [1410

The Scottish Society of the History of Medicine. Report of Proceedings, Session 1958–1959. Pp. 27+1 plate. (Edinburgh: Scottish Society of the History of Medicine, 1959.) [1410

Report of the Hydrographer of the Navy for the year 1958. Pp. 26+4 plates. (H.D. 514.) (London: Admiralty, 1959.) [1410

British Cast Iron Research Association. Thirty-eighth Annual Report for the year ending June 30th, 1959. Pp. 21+5 plates. (Alvechurch, Birmingham: British Cast Iron Research Association, 1959.) [1410

Archives of Oral Biology, Vol. 1, No. 1 (August, 1959.) Pp. i+88. Subscriptions (including postage): (A) For Libraries, Government Establishments and Research Institutions £6 (17 dollars); (B) For individuals who write directly to the Publisher and certify that the journal is for their personal use £3 10*s*. (10 dollars) per annum. (London and New York: Pergamon Press, 1959.) [1410

Philosophical Transactions of the Royal Society of London. Section A: Mathematical and Physical Sciences. No. 1002, Vol. 251 (8 October 1959): The Electronic Absorption Spectra of NH_2 and ND_2. By K. Dressler and D. A. Ramsay. Pp. 553–604+plates 3–5. (London: Royal Society, 1959.) 21*s*. [1410

Council for the Preservation of Rural Wales: Caernarvonshire Branch. Fifteenth Annual Report, 1958–1959. Pp. 22+4 plates. (Plas Hebog, Beddgelert: W. Twiston Davies, Acting Hon. Secretary, 1959.) [2210

University of Bristol. The Annual Report of the Agricultural and Horticultural Research Station (The National Fruit and Cider Institute), Long Ashton, Bristol, 1958. Pp. 188+18 plates. (Long Ashton, Bristol: Agricultural and Horticultural Research Station, 1959.) 15*s*. [2210

Report of the Committee of Enquiry into the Financial Structure of the Colonial Development Corporation. Pp. iii+25. (Cmnd. 786.) (London: H.M. Stationery Office, 1959.) 1*s*. 6*d*. net. [2210

Imperial College of Science and Technology (University of London). Research Report of the Royal College of Science, 1956–1959. Pp. v+77. (London: Royal College of Science, 1959.) [2210

Building Research Station Digest. No. 125 (August 1959): Small Undergound Drains and Sewers, 2. Pp. 5. (London: H.M. Stationery Office, 1959.) 4*d*. [2210

Young Darwin and the Origin of Species. By Dr. C. F. A. Pantin. (Commemorative Oration delivered at the dinner given by the Master and Fellows of Christ's College, Cambridge, in the College Hall on Tuesday, 29 September, 1959.) Pp. 17. (Cambridge: Christ's College, 1959.) [2210

Department of Scientific and Industrial Research. Pest Infestation Research 1958: The Report of the Pest Infestation Research Board with the Report of the Director of Pest Infestation Research. Pp. iv+55+8 plates. (London: H.M. Stationery Office, 1959.) 5*s*. net. [2210

Other Countries

European Productivity Agency of the Organization for European Economic Co-operation. Application of Atomic Science in Agriculture and Food. Vol. 2: Present Position, Future Trends and Techniques—Report of the Working Conference held at OEEC Headquarters, Paris, July 1958. Pp. 247. (Project No. 396.) (Paris: European Productivity Agency of the Organization for European Economic Co-operation, 1959.) 1,300 French francs; 21*s*.; 4 dollars; 14.50 Swiss francs. [810

Annals of the New York Academy of Sciences. Vol. 79, Article 4: Modes and Natural Frequencies of Suspension-Bridge Oscillations. By D. B. Steinman. Pp. 109–142. 2.50 dollars. Vol. 79, Article 5: On the Design of Structural Models to Study the Thermal Stress Phenomenon. By L. Albert Scipio II. Pp. 143–156. 2 dollars. Vol. 80, Article 3: Amine Oxidase Inhibitors. By D. Wayne Woolley and 97 other authors. Pp. 551–1045. 5 dollars. (New York: New York Academy of Sciences, 1959.) [810

Zoology Publications from Victoria University of Wellington. No. 25: Some Additional New Zealand Cephalopods from Cook Strait. By R. K. Dell. Pp. 12. (Wellington: Victoria University of Wellington, 1959.) [1410

Medicina Experimentalis, Vol. 1, No. 1, 1959. (International Journal of Experimental Medicine.) Pp. ii+68. 6 numbers per volume (2 volumes annually). Subscription price per volume: 56 Swiss francs. (Basel and New York: S. Kager, 1959.) [1410

South African Council for Scientific and Industrial Research. Thirteenth Annual Report, 1957–1958. Pp. xvii+226+xix–lix. (Pretoria: South African Council for Scientific and Industrial Research, 1958.) [1410

South Africa. Advisory Council on Scientific Policy. First Annual Report, December, 1956 to 31 December 1957. Pp. 5. (Reprint from *Commerce and Industry*.) Second Annual Report, 1 January to 31 December 1958. Pp. 7. (Reprint from *Commerce and Industry*.) Human Problems in the Scientific Age. By Prof. H. O. Monnig. Pp. 8. (Pretoria: Advisory Council on Scientific Policy, 1958 and 1959.) [1410

Annals of the New York Academy of Sciences. Vol. 82, Article 2: Current Trends in Research and Clinical Management of Diabetes. By Peter H. Forsham and 93 other Authors. Pp. 191–644. (New York: New York Academy of Sciences, 1959.) 4.50 dollars. [1410

New Zealand. Department of Scientific and Industrial Research: Geophysics Division. Seismological Observatory Bulletin No. E 135: January to December 1954, Wellington, other New Zealand Stations, and Fiji. Pp. 27. (Wellington: Government Printer, 1959.) [1410

Kungl. Skogshögskolans Skrifter, Nr. 32: Studier Över Klimatets Humiditet i Sverige. By Olaf F. S. Tamm. Pp. 48+2 plates. (Stockholm: Skogsbiblioteket, Skogshögskolan, 1959.) [1410

Bulletin of the American Museum of Natural History. Vol. 118, Article 5: A Historical Review of the Mollusks of Linnaeus. Part 7: Certain Species of the Genus *Turbo* of the Class Gastropoda. By Henry Dodge. Pp. 207–258. (New York: American Museum of Natural History, 1959.) 1 dollar. [1410

The Carlsberg Foundation's Oceanographical Expedition Round the World 1928–30 and previous "Dana" Expeditions. Dana Report No. 49: Morphologie und Funktion des Kiefer- und Kiemenapparates von Tiefseefischen der Gattungen Malacosteus und Photostomias (Teleostei, Isospondyli, Stomiatoidea, Malacosteidae). Von Klaus Günther und Kurt Deckert. Pp. 54. (Copenhagen: Andr. Fred Høst & Son, 1959.) Danish kr. 15. [1410

The Scientific Film in Germany. By Gotthard Wolf. Pp. 80 (178 black and white and 16 colour plates). (Wuppertal-Elberfeld: Sam. Lucas, Ltd., 1959.) [2210

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LETTERS TO THE EDITORS

ASTRO- AND RADIO-PHYSICS

Directional Observations of Radio Noise from the Outer Atmosphere

ALTHOUGH many observations have been made in the past of the spectra of the radio emissions of the Earth's outer atmosphere in the frequency band 2–40 Kc./s.[1,2], it is only recently that a technique has been developed for continuously monitoring the occurrence of these phenomena[3]. This has revealed that radio noise bursts lasting some hours are normally associated with disturbances of the geomagnetic field and follow many high-frequency radio outbursts from the sun. It seems likely that these very low frequency noise bursts are caused by the interaction between auroral streams of charged particles and the plasma of the outer atmosphere, and proposed mechanisms include Cerenkov radiation[4,5] and gyro- or synchrotron radiation[2]. Studies of their spectra, however, have not provided any clear-cut tests of these theories and it appears that additional information is required.

Many bursts of very low frequency noise have a relatively narrow spectrum, usually about 2 kc./s. wide and centred at about 5 kc./s.[3] It is conceivable that these are caused by synchrotron radiation from particles at a distance of about 6 Earth radii where the geomagnetic gyro-frequency is about 5 Kc./s. In this case the radiation would be guided down the lines of force of the Earth's magnetic field and would enter the ionosphere at geomagnetic latitudes greater than 65°.

Location of the geographical position of the entry point of very low frequency radiation into the ionosphere might therefore be expected to provide a test of the synchrotron hypothesis. This test would be possible if, after penetrating the ionosphere, the radiation spread horizontally in the ionosphere-Earth wave guide. The entry point would then act as a virtual source which could be located by normal direction-finding techniques. However this picture may be incorrect, since it is possible that the radiation spreads horiztonally within the ionosphere and only reaches the ground as an evanescent wave which would not provide useful directional information. Indeed, attempts in the past to locate the entry point of whistling atmospherics with direction finders have been unsuccessful[1].

Before attempting the problem of locating the position and size of the virtual sources of the very low frequency emissions it is therefore necessary to establish that it is possible to identify the direction of the noise. Here we report preliminary results obtained with a direction finder operated at Camden, N.S.W. at a wave-frequency of 4·5 kc./s. The equipment consists of a two 2–50 kc./s. amplifiers connected to two mutually perpendicular vertical loop antennæ each of which is 100 sq. m. in area and has 4 turns. The outputs from the amplifiers are scanned by a rotating condensor goniometer. The system is equivalent electrically to a single loop rotating once every 4 min. The output of the goniometer is fed to a narrow band amplifier (4·0–5·0 kc./s.) and after detection to a minimum reading pen recorder.

When the direction finder is receiving horizontally propagating plane wave radiation its output goes to zero every two minutes when the plane of the equivalent loop is perpendicular to the direction of propagation. If, on the other hand, the virtual source of the radiation is of large extent either in azimuth or elevation, non-zero minimum are recorded, and it can be shown[6] that the ratio of the minimum to maximum output per rotation is a measure of the source size for an assumed source brightness distribution.

Using this direction finder it has been found possible on many occasions to locate the direction and to estimate the size of the virtual sources of the very low frequency radiation at 4·5 kc./s. The source size was calculated in each case by assuming a uniformly bright source distributed in azimuth only. Table 1 shows the results obtained since May, 1959. It can be seen that generally the sources were of large angular size, most being between 30° and 70°. Most directions lay either in the southern or the northern quadrant of the compass, the ambiguity resulting from the use of simple loop antennæ without a sense accessory. However, because of the relatively low geomagnetic latitude of Camden (42° S.) it is considered likely that all virtual sources were to the south. The ambiguities of direction and source brightness distribution will be reduced to a considerable extent in future observations by the use of separate widely spaced direction finders.

Fig. 1*a* shows a sample record of a noise burst recorded at 3/4″ per hr. while Fig. 1*b* shows the corresponding direction-finder record of this burst. The considerable modulation of the direction finding records resulting from the directional properties of the noise is easily visible. Fig. 1*c* shows a record made of another noise burst at a chart speed of 2″ per hr. in order to demonstrate the change in the modulation depth. During this burst the modulation depth increased with time and simultaneously the noise intensity decreased.

It appears from these observations that on many occasions it should be possible to map the positions and sizes of the regions from which very low frequency radio noise from the outer atmosphere emerges below the ionosphere.

Suitable techniques for such a study would include either a network of direction finders or perhaps radio link interferometers similar to those used in radio astronomy.

Table 1. LIST OF DIRECTIONS AND ANGULAR SIZES OF APPARENT SOURCES OF 4·5 KC./S. RADIATION

Date (1959)	Local Time	Minimum Power / Maximum Power	Direction Magnetic	Size in azimuth
May 15	0400	0·16	190°	77°
May 16	1830	0·025	195°	32°
July 11	1800	0·05	205°	44°
July 13	0010	0·40	280°	120°
July 13	0400	0·12	235°	68°
July 13	2100	0·10	120°	62°
July 16	2000	0·25	225°	95°
July 18	2030	0·36	130°	114°
July 18	2200	0·16	140°	75°

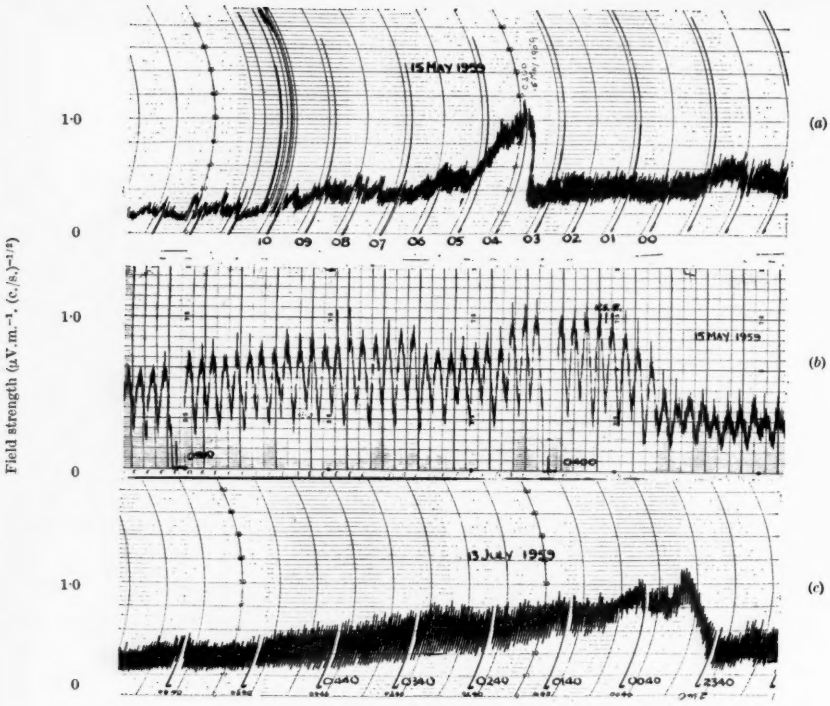

Fig. 1. *a*, 4·5 kc./s. noise bursts recorded at Camden, New South Wales; *b*, direction finding record of noise burst at 4·5 kc./s. showing change in direction finding modulation depth with time; direction finding record of portion of the noise burst of *a*.

Thanks are due to Mr. D. M. Adams for assistance in the construction of the equipment.

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The Inner Solar Corona during June 1959

Study of the solar occultation of the Taurus *A* radio source has yielded new information concerning the structure of the solar corona at large distances[1]; however, the interpretation of the observations is somewhat hampered by lack of knowledge of the electron densities prevailing in the corona at the time of the occultation. Although observations made with the *K*-coronameter[2] at Climax, Colorado, do not extend out to the region of occultation, the measurements in Table 1 should give some indication of the conditions existing in the corona at the time of the recent passage.

In Figs. 1–4 appear polar graphs of the product of polarization p times radiance B of the *K*-corona in units of the radiance of the centre of the solar disk for the dates involved.

To analyse the more complete data of June 10 in detail, we plot as a function of height above the limb: (1) pB averaged at 10° intervals (heliographic) all around the solar limb (Fig. 5); (2) pB averaged at 10° intervals (heliographic) over the south polar region *A* (Fig. 5); (3) pB averaged at 10° intervals (heliographic) over the latitude regions *B* and *C* (Fig. 6); (4) pB at the centre of the active region *D* (Fig. 6). In each case, except the axis of the active region, the variation of pB with height is well represented by the relation found by van de Hulst[3].

Since the Taurus *A* source passes to the south of the Sun, the south polar region is of particular interest. On June 10 the polar region marked *A* was quite weak. Assuming for simplicity in integrating along the line of sight a model with spherical symmetry, we find that electron densities over this region would be about 0·3 times the values tabulated by van de Hulst for a corona at sunspot maximum. Region *A* was flanked by regions *B* and *C* in which the densities were nearly equal and considerably higher than in *A*. On the same assumption, *B* and *C* have densities about that given by the van de Hulst model. The central axis of the active region *D*

Table 1

Date	Scan distances (min. of arc from limb)	Remarks
June 9	2·4	low weight data (calibration several hours later)
June 10	1·9, 4·4, 6·4, 8·4, 11·4, 15·4	standard weight data
June 11	2·4, 4·4, 6·4	low weight data (calibration made in cirrus)
June 17	2·4	standard weight data

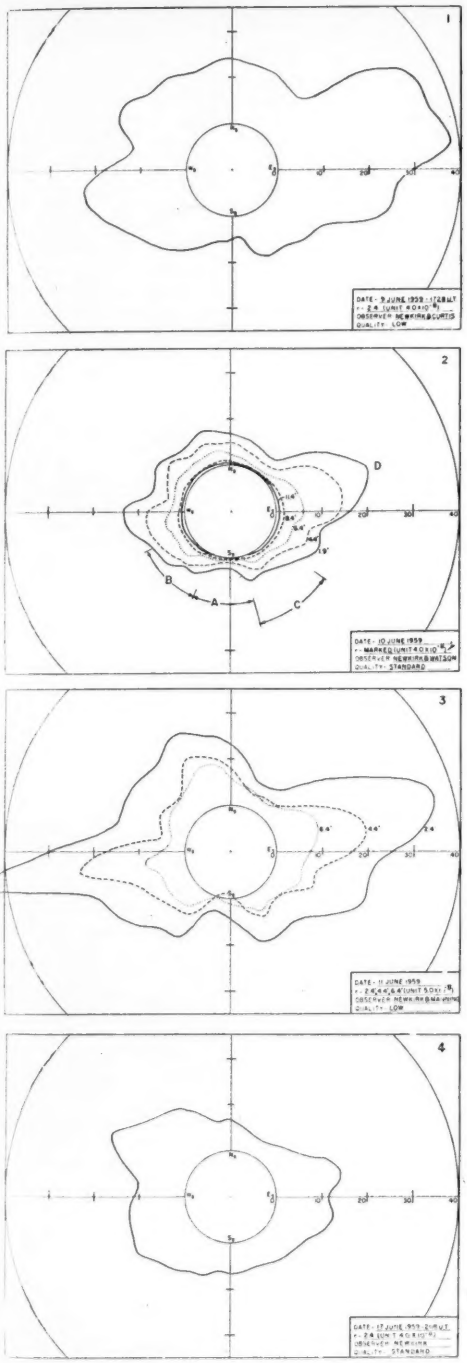

Fig. 1–4. Polar plots of the product pB, where p is the polarization and B the radiance, for the K-corona at the scan heights indicated. The unit is compared to the radiance of the centre of the solar disk and all position angles are heliographic. The scanning aperture is 2·4 min. of arc diameter

is unusual in that the enhancement of electron density seems restricted to the lower 250,000 km. (6 min. of arc above the limb) of the corona. Whether or not this structure is related to the flare and subsequent large loop prominence of June 9 is not known.

Observations of June 9 and 11 are not of sufficient quality to allow more than the determination that the general shape of the corona did not change significantly during the period. By June 17, however, the dip near the south pole had disappeared ; and the electron densities across the south polar cap would have been those of the van de Hulst model if the single scan at 2·4 above the limb can be considered as indicative of the rest of the corona.

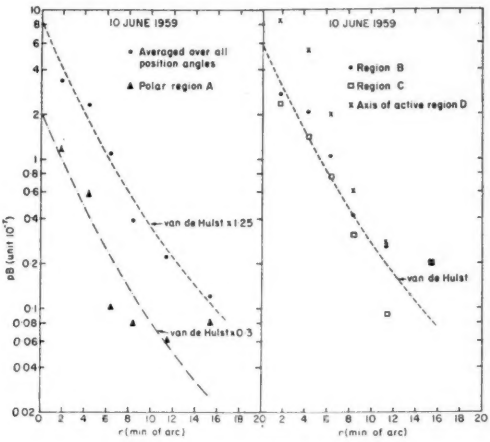
10 JUNE 1959
• Averaged over all position angles
▲ Polar region A
van de Hulst x 1·25
van de Hulst x 0·3
pB (unit 10⁻⁶)
r (min of arc)
10 JUNE 1959
• Region B
□ Region C
x Axis of active region D
van de Hulst
r (min of arc)

Fig. 5 Fig. 6

Fig. 5. Radial dependence of pB for June 10, 1959, for the average of all position angles and for the average over region A (see Fig. 2). At $r = 16$ min. of arc the error in measurement is approximately 60 per cent, while at $r = 2$ min. of arc the error is about 10 per cent

Fig. 6. Radial dependence of pB for June 10, 1959, averaged over regions B and C and along the axis of the active region D (see Fig. 2)

The scanning aperture of 2·4 min. of arc diameter does not allow the detection of small-scale structure such as polar brushes. The tracings do show a ray-like structure in the south-east which has its base at a position angle of approximately 160° heliographic during the interval June 9–11. The feature seems to bend toward lower position angle (equator-ward) at greater distances from the sun.

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GEOLOGY

The Geological Time-Scale

Recent contributions[1–3] on post-Proterozoic geochronology are timely, for national organizations in the U.S.S.R. and in the United States are now preparing reports on this topic for submission to the International Geological Congress next year. In the recent discussions two questions have been raised: (*a*) the validity of the extended time-scale proposed by investigators at the University of Oxford; and (*b*) the validity of age determinations made on the Upper Cambrian kolm of Sweden. On both these issues there is much more evidence than has been cited.

The time-scale proposed by Dr. K. I. Mayne[1] and his colleagues puts back the date of the uppermost Cambrian strata from 450 to 650 million years. The structure of evidence forming the foundation for this conclusion has, deservedly, been demolished by Prof. J. L. Kulp[3] and his associates, of Columbia University; but the latter go too far in asserting that the scale of the Oxford workers "is not supported by measurements other than their own". While for reasons given below I do not accept this scale, it is very relevant that it is upheld by recent determinations reported from the laboratories of the United States Geological Survey[4]. These record a uraninite from Triassic strata in New Jersey giving concordant lead/uranium and lead/lead determinations of 228, 228 and 230 m.y., and a uraninite from Lower Pennsylvanian strata in Pennsylvania giving various ages ranging from 296 to 337 m.y.

This greatly extended time-scale is however ruled out, in my view, by an immense weight of other evidence. The Oxford team claims to have evaluated earlier researches, with rejection of all save eleven determinations, "because the stratigraphy of the samples or their measured age is not free from unwarranted assumptions"; but of their 11 acceptances, which are mostly transgressive igneous rocks of debatable stratigraphy, no less than ten values are rejected by Prof. Kulp. Lately, in preparing a geochronological table to be published elsewhere[5], I have culled from world-wide literature more than two hundred age determinations on Mesozoic and Palæozoic rocks, mostly executed during the past five years. Of these, more than half were adjudged unacceptable because of inadequacies of sampling, analysis or documentation; and the remainder comprises 91 values, all relating to stratigraphically well-defined samples, which cannot be so rejected. Of these values, 66 are derived from Russian literature. The great variety of techniques represented includes rubidium/strontium determinations on micas and glauconite; potassium/argon assays on micas, glauconite, sylvite, primary feldspar, and authigenic feldspar; potassium/argon assays on lavas, tuffs, minor intrusions, slates, hornfelses, and some granitic rocks; potassium/calcium analyses on sylvite; lead/alpha studies on zircon; and helium studies on magnetite. In ten instances two or more methods have been employed on the same sample, with good agreement.

To establish a geochronology from these data without incurring suspicion of subjective selection, an average age has been calculated for the rocks of each system. Where there are sufficient data this should approximate to the mid-point of the period in question. In Table 1 the values derived from recent experiments are compared with the mid-points on the Holmes, Belousov and Oxford scales.

Table 1. Mid-points of the Geological Periods (millions of years)

	Holmes B	Belousov	Oxford	Recent experiments	(Number of records)
Cretaceous	92	90	100	100	(27)
Jurassic ..	140	130	160	153	(9)
Triassic ..	167	169	225	174	(5)
Permian ..	192	205	275	212	(5)
Carboniferous	229	250	350	284	(5)
Devonian ..	284	292	440	329	(11)
Silurian ..	332	328	510	363	(6)
Ordovician ..	390	368	600	410	(8)
Cambrian ..	470	423	700	517	(15)

The records from which these averages were compiled include potassium/argon determinations on feldspars and whole rocks. If there has been loss of argon from feldspar, these ages will be less than the true values. But, notwithstanding Dr. Mayne's conclusions to the contrary[2], the evidence strongly suggests that in unweathered and unmetamorphosed rocks such loss is exceptional. Where sets of analyses are available (in four instances), there is no significant difference between age determinations on biotites, on non-perthitic feldspars, and on whole rocks. It seems that potassium/argon ages on feldspar have quite unjustifiably received a bad name as a result of many demonstrations of loss of argon from pegmatitic microclines. Since Dr. S. S. Sardarov[6] has shown that this loss is directly proportional to the degree of development of perthite or microperthite (thus being dependent on the late thermal history of the rock), we have an acceptable explanation why ages based on pegmatitic feldspars tend to be low, while whole-rock ages on granodiorites, plagiogranites and unmetamorphosed eruptives devoid of perthitic structures agree well with determinations on the biotites which the same rocks contain.

A final word about kolm. Prof. Kulp rejects my contention that although the uranium and lead in the alum shales is syngenetic, these elements are largely epigenetic in the kolm concretions. It would be wise to bear in mind the practical researches of Dr. E. V. Rozhkova and others[7], who have shown that even hydrocarbons as highly anthracitized as the middle Proterozoic shungite of Karelia still retain a marked capacity for adsorbing uranium. Since the groundwaters of the alum shales are, and presumably always have been, highly uraniferous, and since there has been no demonstration that the adsorptive capacity of kolm is out of line with that of similar hydrocarbons, the hypothesis that uranium has been continually introduced into the kolm throughout the ages should not be dismissed so cavalierly. In brief, this material is no more suited to be a geochronological bench-mark than was the uraniferous phosphorite on which Strutt made his pioneer age determinations more than fifty years ago.

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PHYSICS

Sedimentation and Effective Viscosity

In the course of a more extensive calculation, a set of equations has been obtained which relates the the sedimentation velocity u of particles falling through a liquid to the effective viscosity μ of a suspension of similar particles having the same density as the fluid. The volume concentration c is the same in both cases.

To a mixture with mean settling velocity u and concentration c, let us add a particle B the density of which is that of the fluid, that is, it is in suspension. Its mean velocity averaged over all possible positions, is equal to the mean fluid velocity modified by the pressure gradient in the fluid due to the falling particles A :

$$v = v_f + v_p \quad (1)$$

Now assume that its density is increased to that of the other particles A, so that its mean velocity increases to v'. The increase :

$$U = v' - v \quad (2)$$

is caused by the extra external force on it, the forces on the other particles being unchanged. Consequently, if the equations of motion of the fluid are linear, U is also the velocity of fall of B through a suspension of particles A with the same density μ, and, from Stokes's law :

$$U\mu = V\mu_0, \quad (3)$$

where V is the Stokes's velocity of the particle in pure liquid of viscosity μ_0.

Finally, if B is typical of the particles A, its mean velocity of fall is that of the suspension, namely :

$$u' = u \quad (4)$$

Combining the last three equations :

$$\mu u = V\mu_0 + \mu v \quad (5)$$

It is now necessary to estimate the velocity v of B when suspended in the settling mixture. Provided $v << v'$ it can be neglected and we obtain the approximation :

$$\mu u = u_0 V + \text{const.} \quad (6)$$

This is certainly true in the limit $c \to 0$, when $\mu \to \mu_0$ and $u \to V$. It is also an admittedly crude but valid approximation to u for all concentrations.

An approximate value of v, which might apply at low concentrations, can be obtained by neglecting the effect of a pressure gradient and assuming that a suspended particle B moves with the fluid. In a closed vessel the fluid rises as particles fall through it with a mean velocity $-cu/(1-c)$ determined by the equation of continuity. Assuming that :

$$v + v_p = -cu/(1-c) \quad (7)$$

we obtain from equation (5) :

$$\mu u = \mu_0 V(1-c) \quad (8)$$

This approximation seems to agree with the experimental results up to concentrations of about 20 per cent (ref. 1). Above this the value of μu rises fairly rapidly. It is rather surprising that equation 8 holds over such a range of concentrations. In a suspension where the force on a particle is k times its volume, one expects a pressure gradient of the order kc in a closed vessel in the direction of the force, and this corresponds to $v_p \sim \frac{2}{9} cV$ for a spherical particle. This is of the same order of magnitude as v_f and in the opposite dissection and quite large enough to alter considerably the correction factor in equation 8.

As the concentration rises and v_p increases, the value of u should certainly increase, and this agrees once more with experiment. If this analysis is correct, the increase can be used to estimate v_p. Finally, it would be very useful if measurements were made of this drift velocity of a particle suspended in a fluid containing sediment ; they should not be difficult.

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METALLURGY

A New Nitride Precipitate in Iron-Silicon Alloys

Recent work has indicated the presence of a new nitride precipitate in iron-silicon alloys. Leak, Thomas and Leak[1] using internal friction methods investigated nitrided iron-silicon alloys and deduced the presence of an unknown precipitate thought to be an iron-silicon nitride. Turkdogan, Bills and Tippet[2], using X-ray diffraction methods, examined nitrided iron-silicon alloys and found precipitates with an unknown structure which varied with the composition and heat-treatment of the specimen. After the precipitates had been isolated from the alloys by the Beeghly[3] bulk-extraction method, they were found to be α-Si_3N_4 silicon nitride. It was suggested[2] that the precipitates formed in the metal specimens were a complex nitride that decomposed during isolation. With the advent of the extraction replica method, in which included material can be isolated from metal specimens after very mild chemical treatments compared with bulk-extraction methods, a further attempt has now been made to isolate the new precipitate.

A high-purity iron-silicon alloy (B.I.S.R.A. Code No. 33*AF*2) of composition given in Table 1 was

TABLE 1

Silicon	3·05 per cent	Carbon	0·0026 per cent
Manganese	<0·005 per cent	Nitrogen	0·0014 per cent
Sulphur	0·0049 per cent	Oxygen	0·0010 per cent
Aluminium	0·001 per cent	Hydrogen	<0·000005 per cent

(Phosphorus, nickel, chromium and copper were not determined.)

nitrided for 18 hr. at 640°C. and furnace-cooled producing a nitrogen concentration gradient extending for about 0·22 in. inwards from the surface. Much of the nitrided zone had a mottled appearance when examined with the optical microscope. Extraction replicas obtained by the single-etch method[4] after etching for 3 min. with 10 per cent alcohol-iodine solution were examined in the electron microscope and showed the structure to be due to numerous cubic-shaped particles up to about 0·2 μ in size (Fig. 1). Although the appearance of the particles suggested that they possessed a regular crystallographic form, only weak transmission electron diffraction patterns were obtained. The patterns did not appear to correspond to any of the known iron or silicon nitrides and were not identified.

After the above examination, the specimen was further annealed for 6 hr. at 820°C. and furnace-cooled. This caused a considerable change in the appearance of the specimen, the mottled structure being replaced by a coarser, more definite structure. Examination of extraction replicas obtained in a similar manner showed that the structure was due to the presence of rod-like particles identified from electron diffraction patterns as silicon nitride (α-Si_3N_4). The rods occurring in the regions of low nitrogen concentration were few and large (Fig. 2) whilst those at higher concentrations were smaller and more numerous.

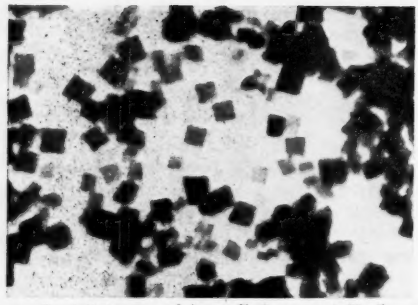

Fig. 1. Precipitates formed in an Fe-3 per cent Si alloy after nitriding and furnace cooling from 640°C. Extraction replica. (Electron micrograph ×20,000)

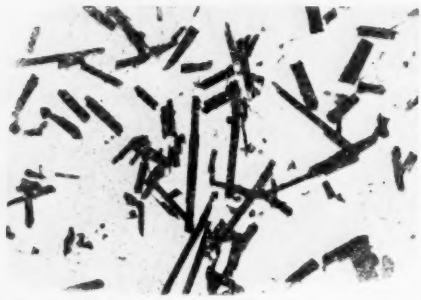

Fig. 2. Precipitates formed in an Fe-3 per cent Si alloy after nitriding and furnace cooling from 640°C., and then further annealing at 820°C. Extraction replica. (Electron micrograph ×2,000)

The results strongly suggest that the cubic-shaped particles are the new precipitate and demonstrate that it transforms within the metal specimen to α-Si_3N_4 when the temperature is increased.

Thanks are due to Mr. R. A. Hacking, director of research, for permission to publish this communication.

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Identification of the High-Temperature Constituent in Mild Steel Surface-Hardened by Carbo-Nitriding

During an investigation on the heat treatment of mild steel in raw town-gas and ammonia atmospheres[1] an unidentified constituent was observed in the surface layers which appeared as a dark-coloured phase visible in the unetched condition. This phase is unstable at room temperature and can be eliminated by slow cooling or reheating. With the limited information available at the time the constituent was presumed to be an iron-carbon-nitrogen compound; positive identification has not been possible until now.

A method of removing thin oxide films from metal surfaces[2] has been adapted for stripping thicker scales [3,4]. A thin plastic film is applied to the surface and the specimen is immersed in an oxygen-free solution of iodine in alcohol, which penetrates discontinuities in the plastic and oxide films and dissolves metallic iron. When the surface deposits have been undermined sufficiently, the plastic film and the oxide particles adhering to it can be removed for X-ray examination. This technique has been applied to machined surfaces, and both sulphide inclusions and cementite lamellæ have been extracted.

The method was used to extract the iron-carbon-nitrogen constituent from a mild steel rod that had been treated for 50 min. at 800°C. in an atmosphere containing 10 per cent ammonia. Before the iodine extraction the specimen was shot blasted to remove any adherent oxides; it was then coated with a plastic consisting of polyvinyl chloride/acetate resin ('Rhodopas *AXCM*') in acetone[3]. After stripping the plastic film was dissolved in hot acetone and the residue collected by centrifuging. When the residue was completely free from plastic it was dried and a small portion was mixed with canada balsam, coated on a hair and mounted in a 19 cm. X-ray powder camera. The photograph obtained with Co-K radiation (Fig. 1) was measured and could be indexed (Table 1) as an hexagonal structure, having lattice parameters $a = 2{\cdot}636$ A. and $c = 4{\cdot}316$ A.

Table 1

d	I	hkl
2·284	m	100
2·158	s	002
2·017	vs	101
1·567	m	102
1·318	m	110
1·218	m	103
1·126	m	112
1·103	m	201

This structure is the same as the ε-iron nitride (Fe_3N) reported by Jack[5]. The parameters are somewhat lower than any observed by Jack but fit well on an extrapolation of his curves to 4·0–4·5 per cent nitrogen (by weight). Jack has also shown[6] that nitrogen in the ε-phase can be partially replaced by carbon and that this reduces the lattice spacing; this indicates that the observed parameters can be accounted for with rather less extensive extrapolation by the presence of carbon. Iron nitrides are notoriously difficult to isolate by preferential solution of the matrix and the successful extraction of this constituent lends support to the inference that it has been stabilized by carbon, and can be identified as ε-iron carbonitride.

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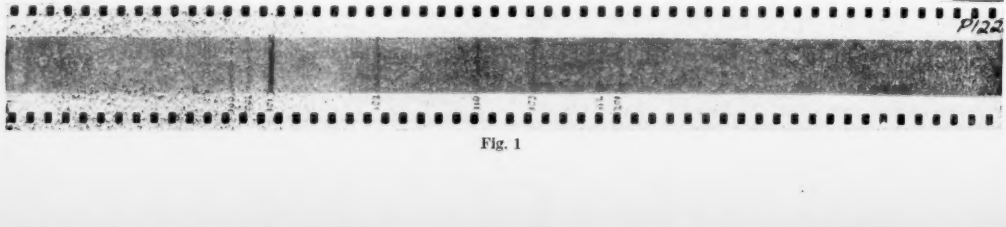

Fig. 1

CHEMISTRY

Effect of Hybridization Changes on the Bond Energies of Carbon—Carbon Single Bonds

It has previously been suggested[1] that the energy of a bond is related to the overlap integral of the two atomic orbitals which are thought to form the bond. To try to provide a quantitative expression of this idea we have assumed that the bond energy is directly proportional to the overlap integral of the bond orbitals. The proportionality constant can be evaluated by using the bond energy of the C_{sp^3} – C_{sp^3} single bond obtained from experimental values of the heats of formation of saturated long-chain hydrocarbons[2]. The bond energies of the five other types of carbon–carbon single bonds may then be calculated by using tables of overlap integrals[3] and the appropriate bond lengths[4] (Table 1). Then by using the C_{sp^2}–H bond energy, obtained from the same set of data as the tetrahedral carbon–carbon bond energy, and the observed[2] heats of formation of ethylene, propylene, acetylene and propyne, the bond energies for the carbon–carbon double and triple bonds and the C_{sp^2}H and C_{sp}–H bonds can be calculated (Table 1). This table of 'standard' bond energies can then be used to predict the heats of formation and heats of hydrogenation of any unsaturated hydrocarbon for which steric effects are small (Table 2). It is seen that the predicted values are quite close to the experimental ones even in cases where there is usually considered to be considerable resonance or hyperconjungative stabilization; in fact, most of the results show a small destabilization energy probably due to the simplifying assumption of neglecting polar effects and non-bonding interactions.

This conclusion that resonance and hyperconjugative effects are small in comparison with changes in hybridization for non-aromatic compounds is in accordance with the recent views[5] that compounds may be classified into two types: (1) those for which two or more classical structures of equal energy can be written, for example, benzene, for which the resonance stabilization is considerable; (2) those for which only one low-energy classical structure may be written, for example, butadiene, cyclopentadiene.

Recent examination of the experimental observations originally put forward as evidence for the predominance of resonance or delocalization effects in governing bond lengths[6], dipole moments[7], force constants[8], chemical reactions[9] and electronic spectra[10] support the view that in type (2) molecules, at least, these effects are small compared with other factors previously ignored.

We should like to record our appreciation for many fruitful discussions with the late Dr. A. Burawoy.

One of us (J. E. B.) wishes to acknowledge the award of an Imperial Chemical Industries Fellowship at the University of Manchester during the tenure of which this work was carried out.

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Table 1. Calculated Bond Energies*

Bond type	Bond distance (A.)	Overlap integral	Bond energy (kcal./mole)
$C_{sp^3} - C_{sp^3}$	1·543	0·647	(82·76)
$C_{sp^3} - C_{sp^2}$	1·530	0·668	85·48
$C_{sp^3} - C_{sp}$	1·460	0·715	91·42
$C_{sp^2} - C_{sp^2}$	1·470	0·716	91·58
$C_{sp^2} - C_{sp}$	1·420	0·754	96·48
$C_{sp} - C_{sp}$	1·380	0·800	103·60
$C_{sp^3} - H$			(98·67)
$C_{sp^2} - H$			98·69
$C_{sp} - H$			102·38
C = C			143·10
C ≡ C			187·23

* Using L_{c-c} = 171 kcal.; $D(H_2)$ = 104·18 kcal.

Table 2. Heats of Hydrogenation (ref. 2) in kcal./mole

Substance	Observed ($-\Delta H$)	Calculated ($-\Delta H$)	Stabilization energy
Ethylene	(32·82)		
Propylene	(30·12)		
Acetylene	(75·06)		
Propyne	(69·70)		
2-Butene (*trans*)	27·62	27·42	−0·20
1 : 3-Butadiene (*trans*)	57·07	55·50	−1·57
1-Methyl-1 : 3-butadiene	54·11	52·80	−1·31
Styrene*	28·20	26·72	−1·48
Stilbene (*trans*)*	20·10	20·82	+0·72
1 : 4-Diphenyl-1 : 3-butadiene (*trans-trans*)*†	44·00	44·62	+0·62
2-Butyne*	65·12	64·73	−0·39
Diphenylethyne*	63·34	59·85	−3·49
Diphenylbutadyne*	126·99	124·67	−2·32
Cyclooctatetraene	98·00	95·84	−2·16
Cyclopentene	26·92	27·38	+0·46
1 : 3-Cyclopentadiene	50·90	51·38	+0·48
Heptafulvene	92·63	96·00	+3·37

* Refers to hydrogenation of aliphathic multiple bonds only.
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Quantitative Paper Chromatography based on the Sub-Micro Titration of Derivatives containing Nitro Groups

An extensive literature on the quantitative paper chromatography of a large variety of organic compounds already exists. Most of these methods are based on: (*a*) measurement of the optical density of a coloured spot produced on the paper chromatogram by spraying with a suitable reagent; or (*b*) measurement of the optical density (in ultra-violet or visible light) of an extract of the component considered, after separation from other components by paper chromatography.

A very simple and rapid method has been developed by us for the quantitative determination of different carbonyl compounds in complex mixtures.

The carbonyl compounds are converted to their dinitrophenyl-hydrazones. The mixture of these derivatives is separated on paper using a modification of the method of Matthias[1]. The bands are cut out, concentrated on a small surface if necessary and put into a small titration vessel containing oxygen-free acetic acid. After addition of a suitable amount of sodium acetate excess 0·003 *N* titanous chloride solution is added (~0·5 ml.) and the mixture stirred for 5–10 minutes (if necessary at about 50°C.). After acidification with hydrochloric acid the excess is back titrated with 0·03 *N* ferric chloride, delivered from a micrometer syringe, using rhodanide as an indicator.

The reduction rates of different dinitrophenylhydrazones proved to be different. The results obtained with a number of dinitrophenylhydrazones after development of a paper chromatogram are summarized in Table 1.

Table 1. Results found by titration of dinitrophenylhydrazones on Whatman-1-paper with titanous chloride: recoveries from 20·0 μgm. carbonyl compound

2·4-Dinitrophenylhydrazone of	Time and temperature of reduction: 5 min. 20°C.	15 min. 20°C.	5 min. 50°C.
Formaldehyde	~16	17·0	20·6
Acetaldehyde		17·0	19·2
Propionaldehyde	~15	19·3	19·4
Acetone	~19	19·9	20·0
Butanone	~16	20·0	
Pentanone-2	16·4		21·2
Cyclohexanone	16·4	~18	19·3
Heptanone-2	20·6	~22·8	24·0*

* Reproducibility excellent

From these results it is clear that a reaction period of 5 minutes at 50°C. will, in general, suffice for the quantitative reduction of the nitro groups. We found that under these conditions the reproducibility is very satisfactory.

Difficulties due to the stability of the titanous chloride solution[2] were completely overcome by proper exclusion of air. In contrast with a statement in the literature[2] we found that light had no influence. A 0·003 *N* solution of titanous chloride in 0·6 *N* hydrochloric acid, stored in a colourless glass bottle did not show a decrease of titre for about one month.

The method described is not restricted to carbonyl compounds. Work is in progress to adapt the method to the analyses of alcohols (dinitro esters), phenols, amines, and amino-acids (dinitro aryl-derivatives). The quantitative reduction of many of these derivatives on paper is possible. The quantitative conversion of the parent compounds to the derivatives is being studied. In principle the method is applicable to all substances containing groups which are reduced by titanous chloride in a reproducible way.

A more detailed description of this work will be published elsewhere.

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BIOCHEMISTRY

Distribution of 5-Carboxymethylhydantoinase

The enzyme 5-carboxymethylhydantoinase, which reversibly cyclized carbamylaspartate to 5-carboxymethylhydantoin, was demonstrated in *Zymobacterium oroticum* by Lieberman and Kornberg[1]. No enzymatic activity was found in rat liver or in two corynebacterium strains[2]. In recent studies on pyrimidine metabolism in man this enzyme was found to be absent in erythrocytes and normal or leukæmic leukocytes[3,4]. The absence of this enzyme in mammalian cells and its presence in bacteria suggested the possibility that chemotherapeutic agents might be developed as competitive inhibitors of 5-carboxymethylhydantoinase. Inhibition of the growth of *Lactobacillus casei* by thiohydantoin-5-acetic acid has been reported[5]. A survey of the distribution of the enzyme in micro-organisms was therefore undertaken.

dl-5-Carboxymethylhydantoin-^{14}C was synthesized from orotate-^{14}C as previously described[3] with specific activity of $1·9 \times 10^6$ c.p.m./μmole. Enzyme assay was based on the hydrolysis of 5-carboxymethylhydantoin-^{14}C (3×10^{-4} moles) to carbamylaspartate-^{14}C during 1 hour incubation at 37°C., *p*H 8·2 (*tris*). Following the addition of 10 μmoles carbamylaspartate carrier and protein precipitation, carbamylaspartate was isolated by elution from a 'Dowex'-1-formate column (1 × 10 cm.) with sodium formate buffer *p*H 3·2 (50 column volumes wash using 0·02 *M*; 20 column volumes using 0·05 *M*). Specific activity was determined in duplicate tubes[3] and the rate of synthesis calculated by the standard carrier formula. *Zymobacterium oroticum* was grown using a modification of the method of Friedmann and Vennesland[6]. Other bacteria were grown in standard laboratory media, harvested by centrifugation, and disrupted by sonication for 10 min. using a Raytheon 10 kc./s. Oscillator. By analogous reactions as for 5-carboxymethylhydantoin synthesis, 1-5-carboxyethylhydantoin was synthesized from 1-carbamyl aspartic acid: m.p. 164–165°C. (dec.) Calculated (per cent): C 41·86, H 4·68, N 16·28; found C 42·07, H 4·91, N 16·39; and 1-5-sulphonylmethylhydantoin from 1-carbamylcysteic acid: potassium salt m.p. 270–274°C. (dec.) Calculated (per cent): C 20·70, H 2·59, N 12·08, S 13·80; found C 20·75, H 2·35, N 12·27, S 13·52.

The results can be summarized briefly. The presence of 5-carboxymethylhydantoinase in *Zymobacterium oroticum* was readily confirmed with rates of synthesis of carbamylaspartate of approximately 13 mμmoles/mgm. protein/hr. Trace enzymatic activities, approximately 1–2 per cent of that in *Zymobacterium oroticum*, were found in *Pseudomonas fluorescens*, *Proteus vulgaris*, and *Staphylococcus aureus*. No significant enzymatic activity could be detected in *Bacillus subtilis*, *Alcaligenes fæcalis*, *Lactobacillus leichmannii*, *Escherichia coli B*, beta hæmolytic streptococcus, *Salmonella st. paul*, Brewer's yeast, or the Ehrlich ascites cell tumour. Neither of the structural analogues were an effective inhibitor of 5-carboxymethylhydantoinase from *Zymobacterium oroticum*. These results and those previously cited demonstrate that 5-carboxymethylhydantoinase has a very limited biological distribution. The metabolic significance of this spur reaction off the general pathway of pyrimidine synthesis has remained obscure, and its limited activity in pathogenic bacteria does not recommend it as a focus for chemotherapeutic attack.

This study has been supported by Grant No. *A*-1606 (*C*2) from the Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service. We are grateful to Dr. H. C. Friedmann for help in obtaining and culturing *Zymobacterium oroticum*.

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Enzymic Conversion of L-Rhamnulose to L-Fuculose in *Escherichia coli*

In a previous paper[1] indirect adaptation to D-arabinose induced by L-rhamnose in some D-arabinose negative strains of *Escherichia coli* has been reported. These strains when grown on L-rhamnose, metabolize D-arabinose and utilize it for growth. This effect is not due to selection of mutants but to enzymic adaptation: induction of D-arabinose isomerase by L-rhamnose has been demonstrated.

This indirect induction can possibly occur through the enzymic conversion of rhamnulose (phosphate) to fuculose (phosphate). Fuculose is known in fact to be an inducer of the metabolic enzymes of D-arabinose[2]. Rhamnulose and rhamnulose-phosphate are normal products of rhamnose metabolism in *E. coli*[3].

Huang and Miller, in their studies on lactaldehyde metabolism[4], also put forward the hypothesis that fuculose was formed from rhamnulose.

We have identified fuculose phosphate among the sugar phosphates obtained by incubating rhamnose with an homogenate of *E. coli* (strain 30) cells in the presence of adenosine triphosphate.

E. coli (strain 30) was grown on a synthetic medium containing 0·3 per cent rhamnose, and the cells were collected during exponential growth. Homogenates obtained by grinding with alumina or by the Hughes press were diluted with 1·1 per cent potassium chloride.

For the preparation of the sugar phosphates, rhamnose was at first isomerized to rhamnulose under the following conditions: rhamnose 0·35 m.mole, homogenate (containing 15–17 mgm. protein per ml.) 8 ml., 0·2 *M* borate buffer 30 ml., cobaltous sulphate, 10^{-4} *M* (final concentration). The reaction was followed by the method of Dische and Borenfreund[5] for the determination of rhamnulose until the equilibrium was reached. At this point 0·35 m.mole of adenosine triphosphate (as the disodium salt), 5 ml. of homogenate and magnesium chloride so as to give a final concentration of 10^{-4} *M* we e added.

After 2-hr. incubation the reaction was stopped with trichloroacetic acid. The precipitate was removed by filtration and the filtrate was brought to *p*H 8. Sugar phosphates were precipitated as the barium salts with ethanol and the precipitate was dissolved in 0·1 *M* acetic acid. Barium was precipitated as sulphate and the nucleotides adsorbed on charcoal until the absorption at 260 mμ disappeared.

Sugar phosphates were chromatographed on Whatman No. 1 paper with 80 per cent ethanol containing 0·8 per cent acetic acid. Two yellow and a green spot were obtained with the orcinol reagent[6]. The green spot was due to free rhamnulose; the others which were due to sugar phosphates also appeared with the ammonium molybdate reagent[7].

The sugar phosphates corresponding to the yellow spots were eluted from paper and hydrolysed with acid phosphatase ('Polidase *S*', Schwarz). The free sugars were chromatographed on Whatman No. 4 paper with benzene–ethanol–water 169 : 47 : 15 v/v using authentic samples of rhamnulose and fuculose as standards.

After hydrolysis, the slow-moving yellow spot gave with the orcinol reagent a yellow and a green spot: the first one had the same R_F and colour as fuculose, the second as rhamnulose. The fast-moving yellow spot gave one green spot moving as rhamnulose.

The sugar moving as fuculose was eluted from the paper. By the cysteine carbazole reaction it gave a red colour with an absorption maximum at 550 mμ. By the orcinol–ferric chloride reaction[8] it showed absorption maxima at 425 and 520 mμ like all ketomethylpentoses. By the Dische and Shettles reaction[9] it showed the 400 mμ maximum characteristic of methylpentoses.

This sugar was also treated with an homogenate of *E. coli* 30 cells containing D-arabinose isomerase. After this treatment, two sugars could be detected by paper chromatography with butanol–acetic acid–water 4 : 1 : 5 v/v as solvent, using the benzidine spray reagent; these sugars showed the same chromatographic behaviour as fucose and fuculose respectively.

Fuculose phosphate, which we have tentatively identified among the sugar phosphates obtained from rhamnose, is probably formed by the inversion of carbon four of rhamnulose phosphate.

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Ionized Calcium in Biological Media

The unsatisfactory status of the important problem of the determination of ionized calcium in biological media has recently been re-appraised by W. F. and M. S. Neuman[1]. After critical examination of methods then existing and of available data on the medium most generally investigated, serum, they state that "the amount of ionized calcium in normal serum now seems to be pretty well established—approximately 1·3 *mM*/litre."

Using a direct general method, quite recently developed, which is based on the absorbance of metal (or *pM*) indicators at two wave-lengths[2], we have redetermined the concentration of ionized calcium in the serum of normal adults using murexide as indicator. The effect of light scattering by serum, and of its variation with the wave-length, is eliminated in this two-wave-length method by the use of serum as a blank at each wave-length. Aniondye binding has also been considered by investigating solutions of Armour serum albumin and apparently is not of significance in this method.

Samples were taken at random from healthy student nurses. Equal volumes of the serums were pooled as a means of obtaining the equivalent of an average normal serum and were analysed almost immediately after being collected. This simple approach was used in order to make a comparison of the results obtained by the new method with those adopted by authorities in the field.

In the following table the values for the formation constants K_f used for the calcium murexide complex correspond[2] to ionic strength 0·15 and the measured *p*H value of each pooled serum (found to be within 0·2 *p*H of the normal).

Table 1

Sample No.	No. of pooled serums	Ionized calcium found (μM/l.)
1	6	1·32
2	2	1·27
3	6	1·29

In addition, serums were collected from hospital patients without obvious metabolic disorders and kept refrigerated for one week. These showed values probably due to alterations of serum on ageing.

There is good agreement between the foregoing results for normal subjects and the value adopted by the Neumans. As emphasized by them, an error of 2 or 3 per cent due to competing ions such as magnesium can be ignored for practical purposes in the frog-heart method. When using spectrophotometry, magnesium does not interfere as in the physiological assay since it remains practically unchelated by murexide in the physiological *p*H-range as previously stated by Raaflaub[3]. This has been verified by us, for we have found a difference of 0·01, at the most, in the absorbances of murexide regardless of the presence or absence of magnesium ions, corresponding to a maximum error of 2 per cent in the value of ionized calcium, which is well within the range admitted in spectrophotometric determinations.

The fact that there is such close agreement between the numerical values for the concentration of ionized calcium in normal serum obtained from methods so different in principle as the new direct method based on *pM* indicators, and indirect methods such as the frog-heart method of McLean and Hastings[4] and the Yendt bioassay technique using the mineralization of cartilage from rachitic rats in human serum as quoted and discussed in the review by the Neumans[1], reinforces the conviction that it is actually the ionized calcium that is determined by all these methods.

Because the usual e.m.f. determinations cannot be used with some of the most important metal ions and since the new membrane electrodes are unsuitable for media containing many different ions, the introduction of suitable new *pM* indicators for the study of metal ions in biological systems seems highly desirable due to the directness and simplicity of purely physico-chemical methods as compared to biological ones.

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Formation of Noradrenaline from Adrenaline by Rat Liver Mitochondria

By condensation with ethylenediamine, adrenaline gives noradrenaline as the main product[1]. This suggests the possibility that adrenaline is demethylated during this reaction.

Conversion of adrenaline to noradrenaline *in vivo* has now been tested in several ways; since this possibility has so far been overlooked except by Lockett[2].

Rat liver mitochondria were shown to convert adrenaline to noradrenaline by the following *in vitro* experiment. The mitochondria (200 mgm., dry weight), prepared by the method of Schneider and Hogeboom[3], were suspended in 5 ml. of $M/30$ phosphate buffer (*p*H 7·0), and L-adrenaline was added to bring the final concentration to 5×10^{-4} M and then incubated at 37°C. for 1 hr. After the incubation, 2 vol. of ethanol containing 2·5 per cent (v/v) 1 N-sulphuric acid were added, and the mixture was kept at 3°C. for 3 hr., then centrifuged and filtered. The filtrate was adsorbed with alumina at *p*H 8·5 then eluated with 0·2 N acetic acid. The eluate was evaporated to dryness under reduced pressure at about 30°C. The residue was extracted three times with 0·5 ml. acid acetone (1 ml. conc. hydrochloric acid in 100 ml. acetone) and the extract was applied to the three filter paper strips (Whatman No. 1)[4]. Phenol containing 15 per cent (v/w) 0·1 N hydrochloric acid[5] was used as the mobile phase.

At the end of run (after about 24 hr.), one of the paper strips was immersed twice in benzene and hung up to dry[4]. When the strip was sprayed with potassium ferricyanide, a red spot appeared at R_F 0·21, which was just identical with that of noradrenaline, together with a spot of adrenaline at R_F 0·52. The quantity of adrenaline was found to decrease compared with controls mentioned below.

When ethylenediamine was sprayed on the second paper strip, a bluish-green fluorescent spot appeared at R_F 0·21 under ultra-violet light.

The zone occupied by the R_F 0·21 substance was cut out from the third paper strip, and was extracted with 0·01 N hydrochloric acid at 3°C. for 12 hr. The extract was examined both by ethylenediamine condensation and trihydroxyindole fluorescence. A part of the extract (5 ml.) was added to a mixture of 0·25 ml. 2 M ethylenediamine dihydrochloride and 0·5 ml. of ethylenediamine hydrate, and the mixture was heated at 50°C. for 1 hr. After the addition of 2 gm. of sodium chloride, the solution was extracted with 2 ml. of *iso*-butanol, and lightly centrifuged[6]. This *iso*-butanol extract was analysed by paper chromatography in the dark, using 5 per cent crystalline disodium hydrogen phosphate, the upper layer of *n*-butanol/ethanol/5 per cent crystalline disodium hydrogen phosphate (50:25:30, v/v)[7], or *n*-butanol-saturated phosphate buffer at *p*H 6·0[8] as the mobile phase. In all cases, the bluish-green fluorescent spot was observed, and the R_F values were identical to the main condensation product of noradrenaline. This condensation product was also examined by paper electrophoresis (0·05 M phosphate buffer at *p*H 7·0, 1 m.amp./cm. of constant current[1]), the mobility of the bluish-green fluorescent substance was identical with that of the main product of noradrenaline.

The extract of the zone of R_F 0·21 substance showed strong fluorescence when it was oxidized by potassium ferricyanide at *p*H 6·0 and then mixed with the combined reagent of sodium hydroxide and ascorbic acid according to Euler and Floding[9].

These results confirm the identity of this substance at R_F 0·21 with noradrenaline.

At the same time, control experiments were carried out as follows: (1) the same amount of the mitochondria was previously heated at 80°C. for 10 min., and then incubated as described above; (2) the same amount of mitochondria in the same reaction mixture was incubated at 0°C.; (3) the mitochondria were incubated at 37°C. with the same reaction mixture without adrenaline. They were treated just in the

same way as in the main experiment. However, no occurrence of noradrenaline was observed.

These results show that noradrenaline was produced from adrenaline by the enzymic action of the mitochondria, which suggests the possibility of the enzymic demethylation of adrenaline.

Considering the different physiological actions of noradrenaline and adrenaline, this reaction is of physiological interest.

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A Bronchodilator Alkaloid (Vasicinone) from *Adhatoda vasica* Nees

A NEW alkaloid has been isolated by us in the crystalline form from the leaves of *Adhatoda vasica* Nees (Indian Patent No. 62349 of November 21, 1957. Patent application No. 64603 of July 9, 1958). The alkaloid, which has been named vasicinone, has been found to be a much weaker base than vasicine, an alkaloid which is already known to be present in this plant. Elementary analysis gave, C = 65·33, H = 4·93, N = 13·65 per cent. The molecular weight (Rast) was found to be about 210 and the molecular formula $C_{11}H_{10}N_2O_2$. The alkaloid was found to be identical with 2,3-(α-hydroxytrimethylene)-4 quinazolone which had been prepared earlier by the oxidation of vasicine with 30 per cent hydrogen peroxide[1,2].

Vasicinone showed characteristic ultra-violet and infra-red spectra and formed salts as well as crystalline double chlorides of gold and platinum. When chromatographed on filter paper (Whatman No. 1) by capillary ascent method using the organic phase of the solvent system obtained from *n*-butanol : acetic acid : water : 10 : 1 : 5, it gave a light red spot when sprayed with Dragendorff's reagent, R_F value = 0·77–0·79. Vasicine under the same conditions gave an orange-red spot, R_F value 0·57–0·58.

It was found that the crude total alkaloids obtained from the leaves of the plant contained vasicine as the main alkaloid mixed with small quantities of vasicinone; but the proportion of vasicinone increased by shaking the crude alkaloids in non-polar solvents like chloroform and benzene and exposing the solutions to sunlight, so much so that after a time, the vasicine in the crude total alkaloids was almost completely converted to vasicinone by auto-oxidation. Pure vasicine could similarly be auto-oxidized to vasicinone.

Vasicinone isolated directly from the crude total alkaloids by partition chromatography (over 'Hyflo', *p*H = 1) was predominantly L-vasicinone and that obtained by auto-oxidation was a mixture of L- and DL- forms. Pure L- and DL- forms could be separated from this mixture. Lævo-vasicinone showed $(\alpha)_D^{32°}$ = −100 (0·5 per cent in chloroform) and melted at 200–201° C., DL-vasicinone melted at 212–213° C. and a mixture of L- and DL- forms melted between 200° and 212° C. Both the L- and DL- forms of vasicinone had similar ultra-violet and infra-red spectra and same R_F value on paper chromatograms.

Recently an alkaloid[3,4] has been isolated from *Peganum harmala* Linn. which has the molecular formula $C_{11}H_{10}N_2O_2$ and melting point 203–4° C. We have confirmed these findings by isolating this alkaloid from the crude alkaloids of the plant and established its identity with vasicinone.

The pharmacological actions of vasicinone on the bronchial musculature were studied on the guinea pig tracheal chain, on perfused guinea-pig lung according to the procedure of Bhattacharya and Delaunois[5], and by the overflow method of Konzett and Rössler[6] in intact guinea pigs. Vasicinone had a definite bronchodilator action on the normal lungs and a powerful bronchodilator action against the histamine-induced bronchoconstriction; but its action was weaker than adrenaline. Lævo-vasicinone was, however, stronger in action than its DL- form. Vasicinone showed a slight and transient fall in the blood pressure of a dog. On isolated perfused hearts of guinea pig and rabbit (Langendorff preparation) vasicinone had a positive inotropic action and increased the flow in the coronary vessels. Both L- and DL- forms of vasicine displayed a bronchoconstrictor action, had a negative inotropic action on the heart and also reduced the flow in the coronary vessels.

The beneficial action of the leaves of *Adhatoda vasica* Nees in respiratory disorders may be attributed to the small quantities of vasicinone, either already present or formed by auto-oxidation of vasicine.

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Interfering Substances in the Determination of Glucosamine Synthesins

PREVIOUS communications have dealt with the enzymatic formation of glucosamine from glucose-6-phosphate and glutamine in cartilage[1,2].

I wished to test the activity of the enzyme involved in glucosamine synthesis in normal and pathological organs. The technique suggested by Castellani *et al.*[1,3] was applied to rat gastric mucosa, aorta, liver, brain, lungs, blood, testis and to rabbit cartilage.

High synthesis of glucosamine was seen when the substrates were incubated with cartilage, liver and gastric mucosa homogenates. To make the determination of activity more specific, the distillation method suggested by Prodi[4], instead of the Schloss method, was used in later experiments. Considerably lower activity values were obtained after distillation, and, moreover, the colour of our samples proved to be due in part to interfering substances, as shown by their absorption spectrum. This fact led me to carry on some hexosamine determination after separation of the interfering substances by means of a cation exchange resin ('Dowex 50'), as suggested by Boas[5].

Table 1. Glucosamine and Interfering Substances Formation in Rabbit Cartilage

Incubated samples		Non-incubated samples		
Interfering substances	Glucosamine	Interfering substances	Glucosamine	Glucosamine synthetized
70	270	23·5	146·4	23·4

Average values of six determinations. Values expressed as γ-glucosamine/gm. fresh tissue. Experimental conditions were those proposed by Castellani and Zambotti (ref. 1).

Still lower values of hexosamine synthesis were obtained by this method, suggesting that the high values given by the Schloss method were due to interfering Ehrlich-positive substances formed or extracted during incubation (Table 1). Part of the interfering substances seems to be due to free glucose-6-phosphate (which gives Ehrlich-positive reaction) liberated from glucose-6-phosphate (which gives Ehrlich-negative reaction), accompanied by the increase of inorganic phosphorus of the incubated samples, as compared to the control samples (450γ free glucose/100 mgm. fresh tissue liberated during incubation with liver homogenates ; 150γ free glucose/100 mgm. fresh tissue liberated during incubation with cartilage homogenate). The incubation of cartilage or liver homogenates with glucose-6-phosphate only, in absence of glutamine, also leads to an apparent synthesis of glutamine.

My experiences suggest that, using the technique proposed by Castellani and Zambotti, in addition to the synthesis of glucosamine, Ehrlich-positive free glucose, liberated by a process of dephosphorylation of glucose-6-phosphate is measured.

I am indebted to Dr. Luigi Tessari for advice and help during the course of the above work.

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Glycolaldehyde Trapped from Aerobic Oxidation of D-Xylose by *Torulopsis utilis*

In previous experiments on the aerobic degradation of D-xylose by living cells of *Torulopsis utilis*, we succeeded in isolating the triose-phosphate, pyruvic acid and the acetyl groups using the phenylhydrazine trapping technique. Our results have been confirmed by Heath *et al.*[3], who purified from *Lactobacillus pentosus* an enzyme which phosphorolytically cleaves D-xylulose-5-phosphate into triose-phosphate and acetyl-phosphate. Schramm and Racker[4] have shown in a mutant of *Acetobacter xylinum* the presence of an enzyme which carries out the same phosphorolytic split of D-xylulose-5-phosphate and cleaves also the fructose-6-phosphate into erythrose-4-phosphate and acetyl-phosphate. From the results of our preceding experiments we supposed a split of an intermediate phosphorylated ketopentose into triose-phosphate and into an unknown C-2 intermediate, both originating the acetyl group according to the formulation given in ref. 2.

The unknown C-2 intermediate was glycolaldehyde, but we were unable to separate it at that time from the trapped intermediates. With chromatographic techniques we have now succeeded in isolating it together with the triose-phosphate, pyruvate and the acetyl group, in the same experimental conditions used in the preceding experiments[2]. Free glycolaldehyde was first isolated by Kaushal *et al.*[5] from the fermentation of pentoses by *Acetobacter acetigenum*. From our results it seems that D-xylulose-5-phosphate, probable intermediate of the fermentation of D-xylose[3], is enzymatically cleaved with the formation of triose-phosphate and glycolaldehyde : the so called 'active glycolaldehyde'. In our aerobic conditions, the acetyl group is formed from triose-phosphate by the way of the pyruvate and from the glycolaldehyde as suggested by us in a preceding paper[6] which deals with the oxidation of acetate to glycolate. In effect, this reaction appears to occur through the intermediate formation of an enolic form of acetyl-coenzyme *A*, which is transformed by hydration into glycolaldehyde, that is afterwards dehydrogenated to glycolic acid.

40 gm. wet weight of living cells of *T. utilis* (Windisch strain), grown on mineral solution at 1·5 per cent of raw saccharose, were washed three times and suspended in the following medium : distilled water 1,000 ml. ; D-xylose (Ciba) 5 gm. ; disodium hydrogen phosphate, 2 gm. ; potassium dihydrogen phosphate, 3 gm. ; ammonium sulphate, 2 gm. ; crystalline magnesium sulphate, 0·3 gm. The *p*H was adjusted to about 5·5, and the suspension aerated in a 1,500 ml. cylindrical glass flask through a sintered-glass disk at the base of the flask. Depending on the *p*H changes, three portions of 1 gm. of the phenylhydrazine oxalate were added within 2 hr. Each portion was dissolved in 50 ml. distilled water containing sodium hydroxide to *p*H 5·5. After 7–8 hr. incubation, the medium was centrifuged and the clear liquid analysed for fixed products[1,2]. For the separation of the glycolaldehyde, in the form of its 2,4-dinitrophenylosazone, the centrifuged medium was treated with an excess of benzaldehyde at 70°C. to free all the trapped intermediates from phenylhydrazine, except the osazone of the glycolaldehyde. The mixture was chilled and filtered to separate the precipitated phenylhydrazone of the benzaldehyde, after which the liquid was concentrated 3:1 and glycolaldehyde isolated by the chromatographic method previously reported[6]. The glycolaldehyde 2,4-dintrophenylosazone obtained melted at 325°C., and no depression was observed in the presence of the synthetic substance; found amounts, 10–70 mgm./l. Failure to trap larger amounts of glycolaldehyde is due to the fact that the aldehyde is degraded also in the presence of phenylhydrazine[6].

Glycolaldehyde was trapped even in experiments on fermentation of L-arabinose by a strain of *E. coli* and one of *Lactobacillus buchneri*. This work is continuing, and a detailed report will be published elsewhere.

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Sugars of the Glycoside of the Root of *Marsdenia erecta* R. Br.

Marsdenia erecta is a plant which grows in Turkey as well as the Near East. Recently some of us[1] have studied the morphological and chemical properties of this plant and isolated a glycoside—marsdenin. To identify the sugar contents of this glycoside and compare with the sugars of the other *Marsdenia* glycosides, we obtained the glycoside, hydrolysed it and identified its sugars by paper chromatography.

200 gm. of dry root of *Marsdenia erecta* were powdered and extracted with petrol ether, chloroform and ether as previously described by F. Korte and I. Korte[2], who used this method for extraction of the glycoside condurangin. The extraction product was dissolved in 200 ml. of 10 per cent methyl alcohol and filtered through an aluminium oxide column. The filtrate is dried by aeration and gave the glycoside. This glycoside is hydrolysed in 20 ml. of 5 per cent sulphuric acid solution in a boiling water-bath for 5 min. and the aglycone fraction is separated by filtration. The filtrate is neutralised with barium carbonate, decolourized with charcoal and dissolved in ethyl alcohol and evaporated: the residue is redissolved in water. The water-soluble hydrolysates were run for 24 hr. on paper chromatograms, Whatman No. 1 (descending technique) using the organic layer from a freshly prepared *n*-butanol-acetic acid-water mixture (4 : 1 : 5, v/v)[3].

These were sprayed with aniline hydrogen phthalate reagent[4] and the chromatograms were dried in an oven at 110° C. The chromatograms showed 4 spots. The first one was dark brown and agreed with authentic specimen of glucose, the second one was brown and corresponded to condurangobiose. Third and fourth spots were dark brown and authentic for thevetose and cymatose respectively.

It seems that the sugars of both glycosides (condurangin and marsdenin) are chromatographically the same. On the other hand the aglycone fractions of these two glycosides are different. To show the difference between these two aglycone fractions, we used different solvents as described by Zechner and Zölss[5], and observed that the solubility of these two aglycone fractions were entirely different.

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Production of Emetic Material by Species of Fusarium

Occasionally crops of barley and other cereals in the mid-western States of America have been infected with one of several species of *Fusarium* that cause a condition known as 'scab'. Such grain often contains an emetic principle[1] which renders it unsuitable for feeding to animals having simple stomachs.

To the best of our knowledge, there are no reports of these micro-organisms producing emetic material in artificial media. As part of an investigation into the physiology of micro-organisms associated with grain, we have found that certain of the *Fusaria* produce emetic material when grown for at least 10 days in a suitable artificial liquid medium with agitation.

The micro-organisms investigated were *F. moniliforme* (two strains), *F. oxysporum lycopersici*, *F. graminearum*, *F. avenaceum*, *F. poae*, *F. sporotrichioides*, *F. equiseti* and *F. culmorum*. All these micro-organisms except *F. oxysporum lycopersici* produce or cause the plant to produce emetic material in grain. Those which produced emetic material in artificial media were *F. moniliforme* (one strain), *F. poae*, *F. culmorum* and *F. nivale*. All the last named, except *F. nivale*, were grown in Richards' solution[2]. For *F. nivale*, which showed poor growth in this medium, nutrient broth was used (3 gm. 'Difco' beef extract, 10 gm. 'Difco' peptone, 10 gm. glucose and 1 litre water). Culture filtrates were evaporated to one-fifth their original volumes, adjusted to *p*H 9 with sodium hydroxide solution and extracted exhaustively with diethyl ether. Upon the evaporation of the dried ether solutions, the ether-soluble residues were examined for the presence of emetic material by injecting an aqueous suspension of 5–10 mgm. intravenously into pigeons. A positive response was indicated by prolonged emesis. Controls prepared similarly by processing sterile media showed no activity.

Work is in progress to ascertain the chemical nature of the emetic compound(s) in these preparations and in extracts of 'scabbed' grain.

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Sterol Glycosides in Oilseed Phospholipids

Sterol glucosides (sterolins) have been shown to be present in the commercial phospholipids obtained from soybean[1], cotton-seed[2], corn[3] and groundnut[4]. Using the acetone extraction procedure already described[4] we have now isolated similar compounds from rapeseed and linseed phospholipids; to the best of our knowledge this is the first report of the existence of sterol glycosides in any part of the flax or rapeseed.

Precipitation with acetone of an ethereal solution of commercial rapeseed 'lecithin' yielded a crude phospholipid which contained 2·1 per cent of sterol glycoside, and a similar substance was found to comprise 2·9 per cent of linseed phospholipids, prepared in a like manner. It is probable that in each case β-sitosterol is the major sterol component, but minor proportions of other phytosterols may also be present; thus detailed examination of the

hydrolysis products from groundnut phytosterolin[4] has revealed the presence of small quantities of stigmasterol and a saturated sterol.

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Isolation of the Antifungal Substance, 6-Methoxybenzoxazolinone, from Field Corn (*Zea mays* L.) in Canada

The presence of an antifungal substance which is inhibitory to the growth of *Fusarium moniliforme* (Sheld.) and *Gibberella zeae* (Schw.) Petch., two pathogenic fungi associated with root and stalk rot of corn in Ontario, has been reported[1]. The present communication reports the isolation and identification of the antifungal substance.

An ether extract was prepared from 9,000 gm. of corn plant tissue by the procedure reported previously. The ether was evaporated and the residue dissolved by boiling in 60 ml. triple-distilled water. On cooling, buff-coloured, needle-shaped crystals formed at 25°C. This crystalline material, at a concentration of 0·12 mgm. per ml. Czapek's agar, prevented growth of *G. zeae*, *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker and Larson, *F. moniliforme* and *Diplodia zeae* (Schw.) Lév.

The crystallization procedure and the type of crystals obtained characterized this material as 6-methoxybenzoxazolinone as reported by Loomis *et al.*[2] and Smissman *et al.*[3] This identification was confirmed by Dr. E. Y. Spencer, Head of the Chemistry Section, Pesticide Research Institute, Research Branch, Canada Department of Agriculture, London, Canada.

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PHYSIOLOGY

Urokinase-Induced Fibrinolysis of Human Standard Clots

Studies with human fibrinolysis, either induced by pyrogens or appearing during thoracic surgery, together with other observations, indicate that very likely the first phase of an endogenous fibrinolytic reaction in man is the release of a plasminogen activator from the tissues into the blood stream. The question whether this activator can induce lysis of an intravascular clot directly was studied *in vitro* with human urokinase, a plasminogen activator excreted with the urine, as the enzyme source and human standard clots as substrate.

Urokinase was prepared as follows: 2,500–3,000 ml. of clear pooled urine was collected during the day, filtered (from inside to outside) overnight by gravity through a Coors porcelain filtering cylinder, porosity No. 1, size 1 (pore size diam. 13·5–15 μ). The next morning, the cylinder was emptied and then eluted by forcing fluids with suction from the outside to the inside in the following succession: 20 ml. of distilled water, which was discarded; 140 ml. of distilled water, which elutes thromboplastic material[1]; 40 ml. of 1 *M* potassium thiocyanate, which was discarded; 120 ml. of 1 *M* potassium thiocyanate, which elutes urokinase. This eluate was cleared by centrifugation and concentrated ten times by pervapouration in a 8 × 32-in. cellulose dialysing tubing. One end of the tubing was dialysed during the pervapouration (6 hr.). Several concentrated eluates, after being cleared by centrifugation and dialysed for 2 hr. against cool running tap water, were pooled and again concentrated by pervapouration (3 hr.). This preparation, 'Uro-100×', was again cleared by centrifugation, dialysed for 90 min. against distilled water and was then ready for use. The activity of the material was retained several days when it was frozen. Fibrinolysis occurred within 20–40 min. when 5 per cent 'Uro-100×' was added to human plasma and the mixture clotted with thrombin.

Standard clots were prepared as follows: Human *ACD*-bank blood plasma was mixed with 10 per cent 0·25 *M* calcium chloride, poured into the stem of a 'Klimax' protein sedimentation tube No. 46815 to exactly the 0·4 ml. mark, and allowed to clot at 37°. At least 1 ml. of the test solution was poured into the wide part of the tube on top of the clot filling the stem. A small glass bead was added, and the tube closed by insertion of a rubber stopper, allowing space for a small air bubble above the test solution. The tubes were chemically clean, siliconized, and the procedure, except for the urokinase, carried out under sterile conditions. The tubes were attached horizontally to a rocking device with a cycle of 30 sec. and a deviation for the horizontal of ten degrees in either direction, and placed in an incubator. The air bubble and the glass bead mixed the enzyme solution gently without touching the clot. With this arrangement, the relation of enzyme solution to the clot and its lysed product was kept constant within a few per cent during the experiment and prevented local accumulation of inhibitor deriving from the lysed clot. There was no marked progressive dilution of the test solution as in the fibrin plate method.

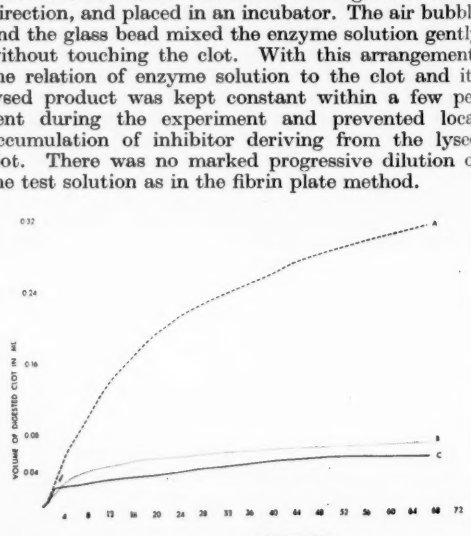

Fig. 1. Continuous fibrinolysis induced in human standard clots by human urokinase. Enzyme solutions: *A*, 10 per cent 'Uro-100×' in buffered saline; *B*, 10 per cent 'Uro-100×' in plasma; *C*, 10 'Uro-100×' in buffered saline, replaced after 2 hr. by saline (arrow). Abscissa; incubation time (hr.); ordinate, volume of clot digested

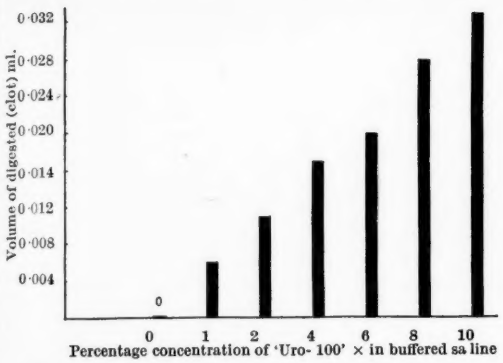

Fig. 2. Fibrinolysis induced in human standard clots by human urokinase. Urokinase replaced by buffered saline after having been in contact with the clot for 5 min. Incubation time, 72 hr. Abscissa, concentration of 'Uro-100x' in buffered saline; ordinate, volume of clot digested

The progress of the fibrinolytic disintegration which started from the one enzyme-covered surface (8 mm.²) of the clot was read directly in volume units of 0·004 ml. and could be followed for many hours.

10 per cent 'Uro-100 ×' in buffered saline produced a continuous fibrinolysis of the standard clot which continued for days (Fig. 1, *A*). 10 per cent 'Uro-100 ×' in human plasma produced a progressive lysis of the clot which was less intensive and slowed down more rapidly than urokinase in buffered saline (Fig. 1, *B*). When the urokinase solution was removed and replaced by buffered saline alone, the clot lysis continued for many hours, but at a reduced rate (Fig. 1, *C*). Urokinase had to stay in contact with the clot for only 5 min., without producing any visible lysis, in order to trigger progressive fibrinolysis when it was replaced by buffered saline alone. The speed of progression was directly proportional to the strength of activity of the contacting urokinase solution (Fig. 2). Replacement of urokinase solution by non-fibrinolytic plasma completely inhibits any further progression of fibrinolysis. Fresh human clots from coronary arteries, femoral veins, and other locations dissolved quickly when exposed to urokinase solutions. From the studies described, the details of which will be reported elsewhere, it is concluded: (1) Orokinase penetrates the clot quickly. (2) Non-retracted clots contain enough plasminogen which, when activated by human plasminogen activator, brings about their lysis. The activator itself may act on the clot *in vieo* and participate directly in thrombolysis.

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Spectral Composition of the Luminescence of the Euphausiid *Thysanoessa raschii*

WE have determined the spectral characteristics of the bioluminescence of *Thysanoessa raschii*.

The crustaceans were collected in Loch Fyne, dark-adapted, deep-frozen, and sent to the Plymouth Laboratory, Marine Biological Association, in vacuum flasks.

For the experiments the animals were homogenized in sea water in a small beaker. The homogenate glowed spontaneously with an intensity that did not change appreciably during the experiments.

The instrument used in the laboratory observations is a telemetering bathyphotometer normally used for undersea photometric determinations. The sensor in the instrument is a R.C.A. 931–*A* photomultiplier tube. The signal from this tube is amplified and fed into a Leeds and Northrup 'Speedomax' recorder. The photocathode is just behind a filter holder, in front of which is a collimating tube. At the distal end of the tube is an opal plastic disk with the characteristics of a Lambert collector. The beaker containing the homogenate is placed at the end of the tube. The light is collimated because narrow-band interference filters are used, and their useful angle is limited to 5°. Twelve interference filters were used in the range 420–640 mμ.

The instrument measured irradiance (H), obtained from

$$n \sim kH \sim sE T_{\lambda} S d\lambda,$$

where n is recorder reading, E is energy of the source through the wave-length interval $d\lambda$, T_{λ} is measured transmission of the individual interference filter through the wave-length interval $d\lambda$, and S is the relative sensitivity of the photomultiplier tube through the wave-length interval $d\lambda$. The value of k was obtained from calibration of the entire instrument against a U.S. Bureau of Standards source. Irradiance values were in watts/cm.², and quanta were derived from Planck's equation. The data were equated to 1·0 at the wave-length of maximum emission.

All measurements were made in a dark room.

The mean of the luminescent emission from four homogenates of *T. raschii* is shown in Fig. 1. There is a sharp primary peak at about 476 mμ, and a slight secondary inflexion can be detected between 500 and 530 mμ. The spectrum is rather similar to that of *Euphausia pacifica*, the only other euphausiid for which an emission spectrum has been determined. The primary peak of the *E. pacifica* spectrum is at 472 mμ when the results are calculated in quanta, and the secondary inflexion is more pronounced than that of *T. raschii*.

Although the 1957 measurements of *E. pacifica* were made with the same instrument, a different series of interference filters was used, and the terminal glow of animals killed with ammonia was measured.

E. pacifica is an open-ocean form, whereas *T. raschii* is confined to neritic habitats.

We thank Dr. Harold Barnes, Millport, for supplying us with specimens of *T. raschii*, and Dr. F. S. Russell for the hospitality of the Plymouth Labora-

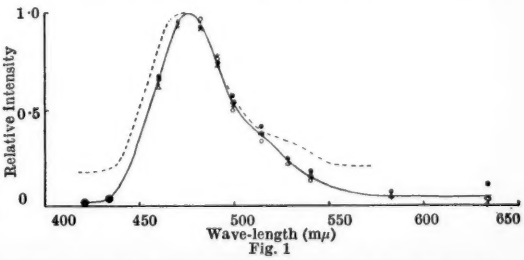

Fig. 1

Fig. 1. Spectral distribution of luminescence from *Thysanoessa raschii* (solid line) and *Euphausia pacifica* (broken line). Data on which the curves were based are in quanta equated to 1·0 at the point of maximum emission

tory of the Marine Biological Association, where these experiments were undertaken. This work received support from the U.S. National Science Foundation.

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Mechanism of Autoregulation of Renal Blood Flow

It has been noted by Winton[1] and has since been confirmed many times[2–5], that the renal vascular resistance is not constant but increases with increasing arterial pressure in the range 80–120 mm. mercury mean pressure. The phenomenon does not depend on an intact nerve supply to the kidney but occurs in the denervated, perfused kidney. The autoregulation of renal blood flow has been considered attributable either to an active vasomotor process[3–5] or, in some recent work, to a flow-dependent separation of plasma from red cells in the renal circulation. The first of these theories appears to have been developed only by the process of elimination, and the second appears no longer tenable in the light of experiments by several groups[5–7]. The œdematous kidney displays a high resistance to flow which probably results from compression of vessels by extravascular fluid. This factor has not been considered important in the function of kidneys subjected to normal arterial and venous pressure, since measurements of renal tissue pressure[5] indicated that it does not vary directly with renal vascular resistance.

In the experiments reported here, kidneys were removed from one dog and perfused with blood from the carotid artery of a second animal. The blood was returned to the second animal's jugular vein. A constant-stroke output pump was used. Its speed was adjusted through a feedback circuit, so that constant pressure was maintained. A series of stepping switches and batteries in the feedback loop made it possible to apply voltage which changed the pump speed and maintained a new pressure. Pressure was measured with a resistance pressure gauge. Flow was calculated from the pump speed and checked with an outflow recorder. Kidney volume was at times recorded by an oncometer or, alternatively, the kidney was suspended from a strain gauge and weighed.

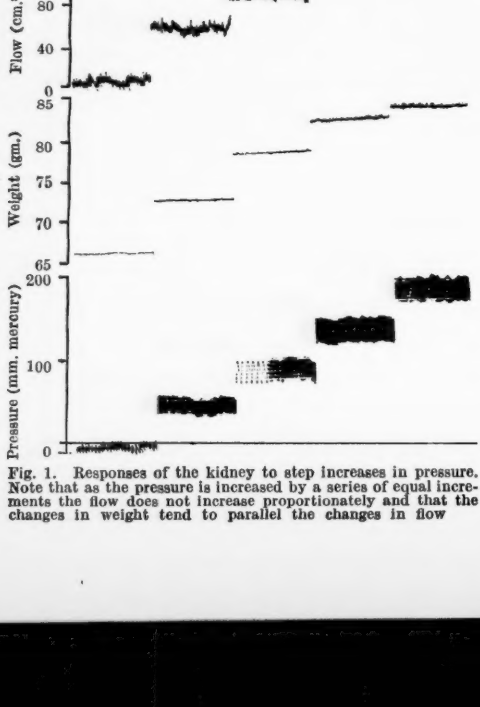

Fig. 1. Responses of the kidney to step increases in pressure. Note that as the pressure is increased by a series of equal increments the flow does not increase proportionately and that the changes in weight tend to parallel the changes in flow

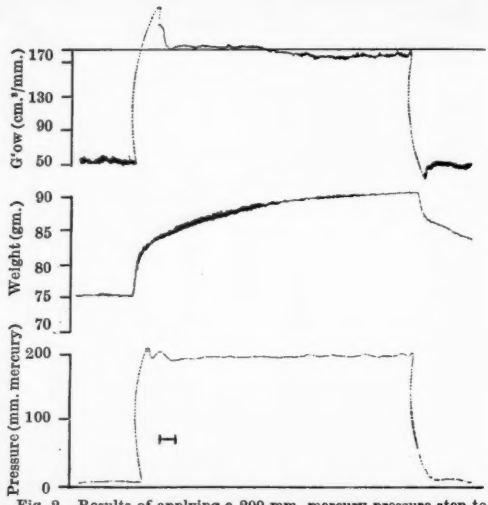

Fig. 2. Results of applying a 200-mm. mercury pressure step to the kidney. The weight changes rapidly at first and then slowly. These two phases are considered to result from vascular filling and filtration of the fluid out of the capillaries respectively. Flow shows an initial increase and then decreases to a final value with about the same time course as the slow pressure change. Time mark below indicates 5 msec.

In 24 experiments, 17 kidneys exhibited autoregulation. The flow always paralleled the renal volume (Fig. 1). When the change of flow per unit pressure change in the autoregulatory range was small, the volume change per unit pressure change was also small. When the kidney was subjected to an abrupt increase in perfusion pressure in the autoregulatory range, the flow rose initially and then fell as the renal volume increased. The increase in volume exhibited a rapid (vascular filling) and a slow (filtration) phase. The equilibrium times for the changes in flow and volume were always nearly identical (Fig. 2). The opposite phenomenon was apparent when the perfusion pressure was rapidly

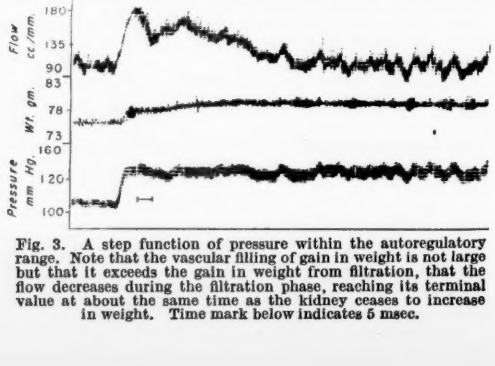

Fig. 3. A step function of pressure within the autoregulatory range. Note that the vascular filling of gain in weight is not large but that it exceeds the gain in weight from filtration, that the flow decreases during the filtration phase, reaching its terminal value at about the same time as the kidney ceases to increase in weight. Time mark below indicates 5 msec.

decreased, that is, the kidney initially had a high resistance which fell as the renal volume decreased.

In some experiments the renal venous hæmatocrit was continuously measured by a conductivity method.[7] As might be expected, the hæmatocrit increased with a rise in pressure and fell with a drop in pressure. This effect was less pronounced in the autoregulatory range than below it, a finding indicating that, in the autoregulatory range, the kidney does not sequester plasma as pressure increases. When autoregulation was abolished by cyanide, the vascular distensibility and the distensibility of the kidney both decreased.

These results conflict with those in which intrarenal pressure did not vary with renal resistance.[8] The fact that renal volume changes parallel renal flow changes is in accord with the possibility that the normal kidney exhibits autoregulation of flow because extravascular fluid compresses some low-pressure vessels. This mechanism has previously been considered unacceptable.[9]

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Carbohydrate Metabolism in Hypervitaminosis A.

Excess vitamin A ingestion increases bleeding tendency[1], depresses basal metabolism[2] and increases excretion of neutral 17-ketosteroids in urine of albino rats[3]. The QO_2 of liver slices of hypervitaminotic A rats is lower than that of control rats (Ray, Amal and Sadhu, D. P., unpublished observations), the weight of the thyroid is diminished and that of the adrenal increased[2]. In an attempt to elucidate the mechanism of hypometabolism induced in hypervitaminosis A, liver was studied for glycogen and fat contents and diaphragm as an index of glucose utilization in peripheral tissues.

Twelve young albino rats of 55 - 60 gm. weight were fed 30,000 I.U. vitamin A ('Arovit' Roche) daily for 10 days and were fed with twelve control rats and were killed by decapitation. A small piece from the upper part of the right lobe of the liver was taken for estimation of glycogen and fat content by a method previously described[4]. The diaphragm was divided into two halves and each hemidiaphragm was used for studying glucose utilization and glycogen synthesis[5] and the values compared with that of the pair-fed control rats. In hypervitaminosis A liver glycogen is decreased from the control value of 15·1 ± 1·8 (standard deviation) mgm. per gm. liver tissue to 12·2 ± 0·93 mgm. while the fat percentage is increased from control 8·1 ± 0·71 to 10·1 ± 0·72. Diaphragms show a decrease of glucose utilization from the control value of 0·31 ± 0·02 mgm. 100 mgm. wet diaphragm per hour to 0·213 ± 0·05 mgm., while glycogen synthesis which is 0·163 ± 0·024 mgm./100 mgm. wet diaphragm per hour in the control rats is decreased to 0·068 ± 0·013 mgm. in the hypervitaminotic A rats.

These experiments show that the depression of metabolism is not restricted to liver alone, but that in muscles is also depressed, in spite of hyperthyroxinaemia in hypervitaminosis[6] A.

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Effect of Thioctic Acid on Gain in Body-Weight by Turkey Poults

Thioctic acid (lipoic acid, DL-6-8 dithiooctinoic acid) is known to be a component of certain enzyme systems. It is also required as a growth factor by *Streptococcus fæcalis* in certain synthetic media, if this organism is to oxidize pyruvate successfully. Certain other bacteria, such as *Escherichia coli*, that oxidize pyruvate also require thioctic acid[1]. It has been tested for its ability to stimulate growth in higher animals; but the results have not been uniform. Positive results were obtained by DeBusk and Williams[2] with rats and chicks. In their experiments a growth response was obtained whether the basal ration was a practical corn-soybean-alfalfa meal type or a purified sucrose–alcohol extracted casein-gelatin type. Feed efficiency was also improved with both the chicks and the rats.

Briggs and Fox[3] afterwards reviewed the literature up to 1957 and initiated another experiment with chicks. They could obtain no evidence of a growth stimulation when semi-purified or practical diets were supplemented with thioctic acid. These workers concluded that thioctic acid could not be considered as an animal growth factor.

Kratzer *et al.*[4] at a later date did obtain a slight growth response to thioctic acid with turkey poults; but the response was not statistically significant. There is a possibility that the turkey poult may differ from the chick in its requirements or in its ability to synthesize micronutrients. The present experiment was set up in an attempt to clarify further the role of this factor in turkey poult nutrition.

A practical poult starter ration, currently in use at this laboratory, was supplemented with thioctic acid at a level of 7 mgm./kgm. This poult starter contained ground wheat and barley, soybean meal, fish meal, meat scrap and alfalfa meal. It was fortified with vitamin and mineral supplements in accordance with general recommendations for this type of diet. Procaine penicillin was added at 9 mgm./kgm. Each dietary treatment was replicated

Table 1. Effect of Thioctic Acid on Poult Growth to Six Weeks of Age

Dietary treatment	Body-weight (gm.)	
	Males	Females
Poult starter	1,152	998
Poult starter plus thioctic acid (7 mgm./kgm.)	1,160	971

six times with 18 unsexed Broad Breasted Bronze poults per replicate. The poults were randomized into the compartments of an electrically heated battery brooder. The experiment was terminated when the poults were six weeks of age.

Body-weight results for male and female poults are summarized in Table 1.

Analysis of variance showed no significant differences in 6-week body weights of male or female poults. The above results would indicate that the response to thioctic acid by turkey poults is negligible when the birds are fed a practical ration.

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A Biological Action of Deoxyribonuclease I on the Growth of *Euglena gracilis*

On realizing that the activity of the enzyme deoxyribonuclease was increased considerably in regenerating rat liver[1], we decided to investigate whether it might influence the rate of cellular multiplication by itself.

In our experiments we endeavoured to maintain the enzyme at the same concentration as it occurs in regenerating tissue. Since nothing is known about permeability of cell boundaries to deoxyribonuclease we tried to compensate for any permeation difficulties. This was done by increasing the outer enzyme concentration by a factor of ten as compared with what we assumed it to be from previous experiments. In the second set of experiments we tested the dose-action relationship.

Euglena gracilis had previously been cultivated for a week in the medium of Elsässer and Adler[2] containing 10^{-10} gm. vitamin B_{12} per ml. 0·5 ml. of this *Euglena* 'suspension' was transferred to 5 ml. of fresh, sterilized medium with vitamin B_{12} contained in 20 ml. penicillin-flasks. The cultures were then incubated in a moist oxygen atmosphere at 28°C. under fluorescent light (Philips *TLSW*). 70 hr. later the total cell volume was determined after spinning down an aliquot at 1,000 *g* for three min. Controls were taken as 100 per cent and the difference in volume was expressed as a percentage increase. It was found that the number of cells was proportional to the total volume of cells within reasonable limits.

Clearly it can be seen from the first experiment that deoxyribonuclease in doses of the order of micrograms will under suitable conditions double the rate of growth in *Euglena*. In the second experiment when the intensity of light is reduced the growth promoting effect is less, but the growth rate is still dependent on the dose of deoxyribonuclease.

Table 1. The action of Deoxyribonuclease I (Worthington) on *Euglena gracilis*

gm. per ml. Deoxyribonuclease I added to culture	Increase in total cell volume or number of cells (%)	Arithmetic mean of increase	Standard deviation	Probability for (ref. 3) perfect random incidence (%)
0·0	12 controls	±0·0	±8·2	>>5
$4·2\times10^{-8}$	12 tests	102·7	±30·7	<<0·5
0·0	5 controls	±0·0	± 5·1	>>5
10^{-7}	−18, −12, −11, 14, 16	−2·2	±14·1	>>5
10^{-6}	6, 9, 9, 14, 16	10·8	± 4·1	<<0·5
10^{-5}	18, 21, 28, 43, 45	31·0	±12·4	<<0·5
$3·3\times10^{-5}$	35, 65, 82	60·7	±23·7	<<0·5

From this experiment it is apparent that the enzyme may act as a growth promoting agent in low concentrations. It will be of interest for us to determine whether this is limited to special organisms or is rather a general phenomenon.

I thank the Deutsche Forschungsgemeinschaft for its support. I am grateful to Dr. Ilse Pendl for supplying a *Euglena gracilis* strain and to Miss. A. Docter and Miss. B. Ohly for their help.

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HÆMATOLOGY

A 'New' Human Blood Group Antigen, Sw_a

In the course of compatibility tests with the serum of a patient Gu., the red cells of one donor, Swann, were found to be strongly agglutinated in all media. The patient was in crisis from auto-immune hæmolytic disease of the 'cold' non-gamma globulin type, with no free 'non-specific' antibody in the serum. Her groups were: 0; cde/cde; NS/Ns, Mi(a−), Vw−, Mg−; P_1+; K−; Le(a−b+); Fy(a+); Jk(a+b+); Wr(a−).

Mr. Swann's groups were: 0; cde/cde, E^w−, C^x−, V−; Ms/Ms, Mi(a−), Vw−, Vr−, He−, Mg−; P_1+; Lu(a−b+); K−k+, Kp(a−b+); Le(a−b+); Fy(a−b+); Jk(a+b+); Di(a−); Js(a−); Wr(a−); Be(a−); By−; Levay−; Rm−. Negative results were also obtained with seven antisera from unsolved 'family' groups, and with over 500 Group 0 (anti-AB) sera. His saliva inhibited anti-H of human and of plant (*Ulex*) origin, anti-Le^a and anti-Le^b, but not anti-A, anti-A_1, anti-B or anti-AB. Nor was the reaction of serum Gu. with his own cells inhibited with Mr. Swann's saliva.

Further testing of the serum Gu. revealed the presence of anti-Mi^a, anti-Wr^a and anti-By. The former was only weak, but the latter two antibodies were avid and powerful, and clearly separable by suitable absorptions both from each other, and from the antibody against Swann's cells. No other example of this latter antibody was found in over 1,200 normal sera, but several examples were encountered in other cases of auto-immune hæmolytic disease. In each such instance, anti-Wr^a was also present, and sometimes anti-Mi^a or anti-Vw as well. Pure antisera were prepared from all these mixtures by appropriate absorption without significant loss of avidity or titre.

It is clear from these observations that a 'new' blood group antigen is present on the cells of Mr. Swann. It is proposed to name this antigen Sw^a, and the corresponding antibody anti-Sw^a. Tests of 29,487 random blood samples from adults disclosed four more Sw^a positives, two of whom were related. Tests on three of the families have shown Sw^a to be inherited as a Mendelian dominant character which

segregates independently of the ABO, Rhesus, MNSs, Kell, Lewis red cell (and ABH secretion), Duffy and Kidd blood group systems; nor is the character partially sex-linked.

These findings will be reported in detail at a later date.

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Glutathione Stability of the Erythrocytes in Iranians

Hæmolytic reactions following the ingestion of drugs such as primaquine and the broad bean (*Vicia faba*) have been shown to be due to an inherited abnormality of the erythrocytes which can be detected by an *in vitro* glutathione stability test devised by Beutler[1]. The use of this test in surveys has established that the incidence of the defect varies with peoples and race. American Negroes, Sephardic Jews and Sardinians have a much higher incidence of sensitive individuals than Ashkenazic Jews or American Caucasians. Beutler has recently reviewed the subject[2]. Many cases of favism have been seen locally during the past few years, and it was reasonable to assume that a survey of the glutathione stability of the erythrocytes in Iranians would show the presence of the abnormality in this area.

The survey group consisted of 556 Moslems who were members of the medical, nursing and ancillary staff at our two institutions. Only one member of a family was sampled. However, because consanguinity is quite common in Iran, it was impossible to be sure of eliminating children of first-cousin marriages. Persons of Jewish, Armenian, Assyrian or Zoroastrian origin were not in sufficient number for evaluation. These groups, along with various tribes, are being studied, and will be the subjects of later reports. The blood samples were collected into an acid-citrate-dextrose solution; glutathione determinations and the glutathione stability test were performed by the standard technique usually on the same day or within 24 hr. of collection. Hæmatocrit values were also determined.

Since it has been shown that the gene which controls the abnormality is probably sex-linked, the results were calculated independently for the two sexes. Using as the criteria of sensitivity to the glutathione stability test all values of less than 30 mgm. glutathione per 100 ml. red blood count after incubation with acetylphenylhydrazine[3], there were 35 sensitive males out of 358 (9·8 per cent) and 12 sensitive females out of 198 (6· per cent). This difference of incidence between the males and females in this series is not statistically significant ($P > 0{\cdot}10$). Table 1 gives an analysis of the levels of glutathione in the erythrocytes before and after incubation with acetylphenylhydrazine and Table 2 shows the distribution of post-incubation glutathione-levels among the sensitive individuals. As previously described by other workers[4], sensitive males gave lower post-incubation glutathione-levels than females.

Table 1. Analysis of Blood Glutathione-levels before and after incubation with acetylphenylhydrazine (the glutathione stability test)

	No. of subjects		Range of Values	Mean	*S.D.*	*S.E.*
Sensitive males	35	before	23·2– 70·1	33·9	8·3	1·4
		after	0·2– 19·6	5·1	4·9	0·8
Nonsensitive males	323 (257)*	before	40·0–111·5	64·1	12·3	0·8
		after	33·7– 95·5	54·6	12·5	0·7
Sensitive females	12	before	26·9– 59·6	43·7	11·0	3·2
		after	0·7– 27·1	14·2	9·4	2·7
Nonsensitive females	186 (132)*	before	36·3–124·0	66·6	15·6	1·4
		after	32·8–109·2	60·5	13·2	1·0

All values are in mgm. glutathione per 100 ml. red blood cells.
* The numbers in brackets are the number of samples tested both before and after treatment with acetylphenylhydrazine; the remainder of the nonsensitive persons were tested only after treatment with acetylphenylhydrazine.

Table 2. Distribution of glutathione-levels (in mgm. glutathione per 100 ml. red blood cells) amongst sensitive persons after incubation with acetylphenylhydrazine

Range of values	0·1–5·0	5·1–10·0	10·1–15·0	15·1–20·0	20·1–30·0
No. of males	22	8	3	2	0
No. of females	2	3	1	0	6

Having thus established an overall incidence of 8·5 per cent of sensitive individuals, the subjects were analysed according to place of birth. The results were as follows: Shiraz area 275, rest of Fars (the province in south-west Iran of which Shiraz is the chief town) 89, Bushire 5, Teheran region 53, Azerbaijan 8, Caspian sea area 12, Khohrasan 15, Kerman 4, Kurdistan 12, Bandar Abbas 1, Khuzistan 18, Yazd 26, Isfahan and province 31, born of Iranian parents outside Iran 7. Of the total of 47 sensitive persons, 26 were born in Shiraz (that is, an incidence of 9·5 per cent in this area), 10 were born in the rest of Fars and 11 were born in the rest of Iran. Though the number of subjects born outside Shiraz constituted slightly more than half of the series, they were too spread out over Iran to allow comparison with the Shiraz group. However, it appears that persons sensitive to the glutathione stability test are to be found in the Moslem population throughout Iran.

At the same time as this survey was performed, a number of other genetically determined characteristics were also examined. A statistical analysis of the ABO and Rh data revealed that the survey group represented a good random selection of individuals. The results of these and other blood anthropological studies will be presented later.

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Hæmoglobin Pattern of the Cyclostome *Petromyzon planeri* during the course of Development

Two hæmoglobin components have been demonstrated by starch gel electrophoresis in both larval and adult forms of *Petromyzon planeri*[1]. Although the components are most clearly separated by electrophoresis in starch gel, separation has also been

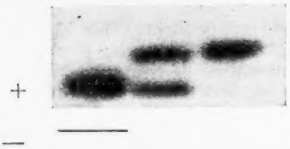

Fig. 1. Re-migration of the single hæmoglobin bands from larvæ of *Petromyzon planeri*. The slow and the fast components (on the sides) were split by electrophoresis on starch gel and then eluted and run again together with another sample of the original hæmolysate (in the middle). Starch gel electrophoresis at *p*H 8·8 (*tris*-borate system of Poulik, 2·5 V./cm. for 14 hr.)

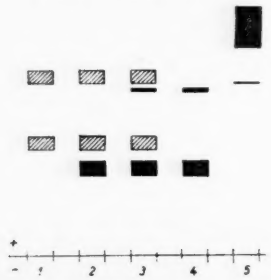

Fig. 2. Electrophoretic patterns of lamprey *Petromyzon planeri* hæmoglobins. Size of the rectangles in the diagram is proportional to the intensity of the hæmoglobin bands after staining with benzidine : 1, typical larval pattern between May and December ; 2, 3, patterns observed between January and March mostly in individuals showing obvious signs of metamorphosis ; 4, typical adult pattern from May onwards ; 5, human hæmoglobin pattern in the same experimental conditions. (Conditions of the experiments as described under Fig. 1.)

obtained by starch block electrophoresis and on acetate cellulose paper. Furthermore, after elution of the individual bands it was found that each one then runs as a single component in the starch gel system (Fig. 1). It seems therefore quite likely that the separation in the different hæmoglobin bands is due to differences in molecular size. Concerning the electrophoretic mobility of these hæmoglobins as compared to human ones it must be noted that their isoelectric point, unlike that of other species of lampreys[2], seems to be in the region of neutrality.

All the larvæ and adults collected between April and May 1958 from the River Sarno (Naples) show only two such hæmoglobin components, but in the larval form each of them has an appreciably greater mobility than the corresponding one in the adult forms. Furthermore the two components are present in about the same amount in the larvæ, while in the adults the fast one is always very faint as compared to the slow one. It has now been found that if the material is collected between January and March, several individuals are observed which possess three, or all four, components (Fig. 2). This is a period when metamorphosis is in progress and the specimens with three or four hæmoglobin components are much commoner among individuals showing obvious sings of metamorphosis.

In late April and May all individuals found in Nature are either of the adult or of the larval type, and no animals with more than two hæmoglobin components are found. Later on the sexually mature individuals die after spawning. Only ammocœtes survive and they show the larval hæmoglobin pattern until the beginning of the following winter. It is conceivable that the changes in the pattern may be of some physiological importance, possibly connected with metamorphosis and sexual maturation.

It should, however, be noted that apart from the gross morphological and physiological changes, which take place at metamorphosis, there is also a corresponding change in the ecology of the organisms : the adults swim freely in the river, the larvæ remain in the mud of the bottom. At the present stage, however, the significance of the difference in character of the hæmoglobin between the larvæ and the adult remains still uncertain.

Finally it must be added that there was no obvious evidence of genetic polymorphism in the hæmoglobin pattern of either the 119 ammocœtes or the 102 adults from the River Sarno so far investigated. The same adult type hæmoglobin pattern was observed in adult individuals of *Petromyzon fluviatilis*, a species closely related to *Petromyzon planeri*, which lives in the sea during the early larval stage and comes up to fresh water to reach maturity.

Further investigations are now in progress to characterize the structure and the properties for each of the hæmoglobin components described above, bearing in mind what is already known of the hæmoglobins in lampreys[2,3] and in other Cyclostomata[4].

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PATHOLOGY

Antileukæmic Activity of Polyporic Acid

In the course of screening indigenous plant products for experimental anti-cancer activity, a preparation of the lichen *Sticta coronata* was found to prolong significantly the life-span of mice previously inoculated with an acute lymphocytic leukæmia. Polyporic acid (2,5-dihydroxy 3,6 diphenyl 1,4 benzoquinone) isolated from *Sticta coronata* was shown to possess antileukæmic activity and to account completely for that of the original crude extract. Synthetic polyporic acid shows similar antileukæmic activity to that of the naturally occurring compound, and no reference to antitumour action has been encountered by us.

The acute LD_{50} (intraperitoneal) is 80 mgm./kgm. for *C3H/DBA2/F1* hybrid mice maintained on a *41B*-type diet. Antileukæmic activity is observed in the dose range 50–70 mgm./kgm. the upper limit of which causes weight loss and occasional deaths among treated mice prior to the occurrence of leukæmic deaths in the control groups.

Hybrid mice inoculated with one million cells of the acute lymphocytic leukæmia *L1210* show 50 per cent survivals of 6·5–7 days or of 9 days, when the leukæmic inoculum is made intraperitoneally or intramuscularly, respectively. Daily intraperitoneal dosage with polyporic acid at 60 mgm./kgm. commencing 24 hr. after tumour implantation results in extension of 50 per cent survival times to about 13 days. Regardless of the differing 50 per cent survival times of control animals inoculated with leukæmic cells by intramuscular and intraperitoneal routes, that of treated animals in both groups is similar.

Sarcoma 180 ascites accumulates more slowly in Swiss albino mice treated with polyporic acid at 60 mgm./kgm. than in saline injected controls. In a typical experiment, seven days following tumour

inoculation treated mice contained an average of 0·7 ml. ascites (0·1–2·9 ml.) whereas control mice had 3·8 (1·6–5·8) ml. In each case hæmatocrit determinations showed an average packed white cell content of 2·5 per cent approximately.

No significant growth restraint was produced in a transplanted *C3H* mammary cancer in mice treated parenterally with 60 mgm./kgm. polyporic acid for periods of 14 days.

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Sporidesmium Bakeri Recorded from Victoria, Australia

Strong evidence was produced[1,2] in 1958 that facial eczema of sheep in New Zealand is caused by ingestion of spores of a fungus identified as *Sporidesmium bakeri*. Lately I carried out experimental work in New Zealand during January–March, 1959, which involved *inter alia* the isolation of *Sporidesmium* spores from pasture. On return to Australia a search made of pastures in southern Victoria disclosed the presence of a fungus which appeared superficially identical to the New Zealand *Sporidesmium*. A culture was forwarded to the Commonwealth Mycological Institute, who report that it is indeed *Sporidesmium bakeri*.

Some three years ago a minor outbreak of a disease similar to New Zealand facial eczema was reported from certain areas in south-eastern Victoria. Within the past few months a much more serious outbreak of what definitely appears to be facial eczema has been reported from a number of properties in this area. This outbreak appears to have followed an unusually dry summer followed by a rainy period, conditions similar to those which lead to facial eczema outbreaks in New Zealand. The finding that the same fungus exists in Victoria supports the possibility that the two diseases are identical, and the isolate is being administered to sheep as a preliminary check.

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[1] Percival, J. C., and Thornton, R. H., *Nature*, **182**, 1095 (1958).
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Sporidesmium bakeri and Facial Eczema of Sheep in the Field

This communication records a relationship between the occurrence of *Sporidesmium bakeri* and facial eczema disease of sheep in the field. It has already been demonstrated that experimental animals fed cultures of this fungus developed characteristic lesions of the disease[1,2].

At the Manutuke Research Station, Gisborne, a facial eczema research farm of the New Zealand Department of Agriculture, two, 1-acre fields (No. 20 and No. 31), of perennial rye grass, white clover have been closely mown at 7–14 day intervals, with motor lawn-mowers since the spring flush of pasture growth in October, 1958. In field No. 20, the mower clippings were returned to the pasture by the expedient of leaving the catcher off the mower, while in No. 31 the catcher was attached and the clippings were removed. Neither field was grazed from October, 1958, to January, 1959, and under this mowing treatment both fields had a high proportion of white clover in the sward.

In field No. 20 there was an accumulation of litter resulting from the return of clippings and in early January, 1959, *S. bakeri* was observed to be growing and sporing profusely on litter and dead grass and clover leaves. Spores were found to blow in the wind, particularly when the surface of the ground was disturbed, and microscopical examination revealed spores adhering to the leaves of living grass and clover leaves. Thus spores on both dead and live plant material would be ingested by stock. In field No. 31 little was sparse and spores were present in lower numbers.

Field No. 10 was employed as a control and was a typical, grazed summer pasture, 4–6 in. long and containing a high proportion of old dead ryegrass stalk and leaf, in which spores were scarce and difficult to detect.

Spore counts were made on pasture samples cut with hand shears ½ in. above the ground. Fresh pasture (25 gm.) was shaken for 1 min. in tap water (250 ml. plus one drop 'Teepol'), and filtered through a 2-mm. sieve. Spores in the filtrate were counted with a Neubauer blood cell counting chamber. 10 samples of grass were collected in each field, 5 equally spaced along each of two diagonals across the field. Spores in five 1 sq. mm. squares (centre and four corners) were counted in each of two counting chambers prepared for each sample, and counts expressed as mean values. Statistically the accuracy and reproducibility of this method was good. The results recorded in Table 1 do not necessarily indicate the total spore contents of the pastures as the percentage recovery of spores by this technique is not known.

Table 1. Counts of Spores of *S. bakeri* per 1 sq. mm. Counting Chamber

Field number and treatment		Sampling date : January, 1959			
		9	16	22	27
20 Mow and return clippings	.. ..	3·3*	2·8	6·03	3·68
31 Mow and remove clippings	.. ..	0·33*	1·06	1·2	1·76
10 Control	.. ..	present only	present only	—	0·13

Spores per gm. fresh-weight pasture are obtained by multiplying these figures by 10^5

* Results from bulk samples.

The increase in spore count in field No. 20 on January 22 was due to the colonization by *S. bakeri* of fresh clippings added to the pasture when the field was mown on January 16.

On January 9, five shorn Romney wether lambs were placed in each of fields Nos. 20 and 31 on a basis of paired live-weights, and 10 lambs were put in field No. 10. These lambs were judged to be free of facial eczema at the time because twelve lambs from the same flock, killed a day earlier showed no evidence of liver damage. The lambs in field No. 20 showed early symptoms of photosensitivity on January 16, and by January 26 three had developed facial lesions. In field No. 31 one lamb developed facial lesions on January 27, and on this date all five lambs appeared to be suffering

from acute photophobia as they sought the shade at every opportunity. On January 28 the lambs in fields Nos. 20 and 31 were slaughtered and in each case extensive liver damage characteristic of facial eczema was observed. This grazing trial was immediately repeated with a further five lambs in each of fields Nos. 20 and 31 during the following four weeks, and very similar results were obtained although the degree of liver damage appeared even more severe. Spore counts remained at similar levels over this further period.

Lambs grazing over the same periods on field No. 10, and on other fields, in which spore concentration was relatively much lower, showed no evidence of photosensitivity and only a low incidence of mild liver damage was seen in lambs which were slaughtered.

This evidence establishes a relationship in the field between facial eczema disease of sheep and *Sporidesmium bakeri*.

The fact that spore counts were low in field No. 10, where there was a high proportion of old dead rye-grass stalk and leaf in the 6-in. long pasture, and were relatively high in the regularly mown fields Nos. 20 and 31, where pasture was much shorter, suggests that freshly killed or freshly damaged pasture plants, and the length of pasture, as affecting microclimatic conditions, were important factors in inducing a high spore-count.

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Fig. 1. Agar-gel diffusion tests with serum of Taylor Central-well containing serum : 1. *T. rubrum* cell-sap ; 2. *T. rubrum* culture medium filtrate ; 3. *C.herbarum* extract ; 4. *T.mentagrophytes* cell-sap ; 5. *T.mentagrophytes* culture medium filtrate ; 6. *P.notatum* extract.

Fig. 2. Agar-gel diffusion tests with serum of Shaw. Central-well containing serum : 1. *T. rubrum* cell-sap ; 2. *T.mentagrophytes* cell-sap ; 3. *A.fumigatus* cell-sap ; 4. *C. herbarum* extract.

Human Precipitins against Common Pathogenic and Non-Pathogenic Fungi

The demonstration in human sera of precipitating antibodies against common pathogenic and non-pathogenic fungi is of interest for the study of fungal antigens and for the study in man of reactions of the Arthus type which are dependent upon the presence of precipitins. Reactions of this type have been reported[1] in patients with precipitins against members of the *Aspergillus* genus. Certain of these sera also contained precipitins against *Cladosporium herbarum* and *Penicillium notatum*, and common antigens were demonstrated in them and in the *Aspergillus* genus.

We now wish to report the presence in human sera of precipitins against the common dermatophytes, *Trichophyton rubrum* and *Trichophyton mentagrophytes* var. *interdigitale*. The antigens were prepared both from the minced fungal mycelium and from the culture medium (Sabouraud's peptone-glucose broth), which were dialysed against running tap-water for 24 hr. freeze-dried and reconstituted in 0·5 per cent carbol-saline in concentrations of 10 and 20 mg./ml.

Agar-gel diffusion tests were performed on the serum of patients with proved *T. rubrum* infection and on other sera some of which were known to contain *Aspergillus* precipitins (Table 1). No information was available regarding past or present *Trichophyton*

Table 1. Agar-gel Diffusion Tests on Sera containing Various Known Precipitins

No. of patients	Precipitating antibody against :						
				T.rubrum		*T.mentagrophytes*	
	A.fumigatus	*C.herbarum*	*P.notatum*	Cell-sap	Culture medium filtrate	Cell-sap	Culture medium filtrate
14 (*T.rubrum* patients)	0	7	0	14	14	14	14
7	7	6	4	5	5	5	5
55	0	1	0	47	3/5 tested	5	0/5 tested*

* The sera in this group were tested at a later date than the other two. The low incidence of reactions to *T.mentagrophytes* may be due to modification of its precipitating antigens by tryptic enzymes, present in the preparations from both species.

infection in the patients concerned in the latter group.

In some tests at least three precipitation lines were produced by the *Trichophyton* antigens, though the commonest finding was a broad, sometimes dense, precipitation band, in which on occasion clearly defined precipitation lines appeared on standing for 10–14 days. Sharply defined precipitation lines were obtained with the *Trichophyton* antigens against sera known to contain *Aspergillus* precipitins. This is clearly shown in Fig. 1 (serum of Taylor).

The cell-sap and culture medium preparations from *Trichophyton* cultures contained common antigens. Additional antigens were found in the cell-sap of *T. mentagrophytes* var. *interdigitale* which were not present in the culture medium filtrate. This situation was reversed in the case of *T. rubrum*, additional antigens being found in the culture-medium filtrate. The precipitation lines produced by these additional antigens lay farthest away from the central antibody containing well. This is shown in Fig. 1, in which the cell-sap of *T. mentagrophytes* var. *interdigitale* has produced an additional precipitation line. This serum (Taylor) also contained precipitins against *C. herbarum* and *P. notatum*. Common antigenic determinants were found in *T. rubrum* and *C. herbarum*, and in *T. mentagrophytes* var. *interdigitale* and *P. notatum*. In tests on another serum containing precipitins against *A. fumigatus* it can be seen that *T. rubrum* and *A. fumigatus* possess common antigenic determinants (Fig. 2).

The addition of 2–5 mgm. of freeze-dried *Trichophyton* antigen to 0·1–0·2 ml. of the serum inhibited the precipitation lines of *Trichophyton* and *C. herbarum* antigens partially or completely, depending on the amounts added. Addition of the same high concentrations of *A. fumigatus* antigen to the sera inhibited the formation of precipitation lines by the *Trichophyton* and *C. herbarum* antigens. These tests confirmed the presence of common antigenic determinants in these fungi.

The precipitins reported here should be of value in elucidating the complex pattern of antigenic relationships between these fungi, and have the advantage of occurring in human sera.

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BIOLOGY

Chromosomal Evolution in Eutherian Mammals

This communication is an abstract of a longer paper which will be published in the *Archiv der Julius Klaus Stiftung für Vererbungsforschung*. It gives the conclusions I have reached by applying statistical techniques to the problem of the evolution of the chromosomal formulæ of eutherian mammals.

A critical check-list of the chromosome numbers gives the data for 240 species, 135 of which belong to a single family, Muridæ. This sample represents about 8 per cent of the described species, the proportion being the same for eutherians as a whole and for Muridæ. The latter form a homogeneous group, the comparative chromosomal cytology of which is fairly well known. As against other systematic categories which are being actively studied, such as Drosophilidæ or Saltatoria, these rodents present the following advantages: a comparatively recent origin, an intense adaptive radiation, highly differentiated chromosomal formulæ.

Since mammal orders have been very unevenly studied, some of them, for example, Proboscidea, Hyracoidea, Dermoptera, not at all, the Muridæ sample is more representative than the total eutherian one. The results obtained, however, are very similar for the two samples.

The statistical analysis based on the 'fiducial limits' method allows us to draw the following conclusions: There is a 95 or 99 per cent probability that 55–68 or 53–70 per cent respectively of the eutherian species have diploid numbers between 40 and 56. For Muridæ these percentages are much the same: 59–76 (5 per cent confidence-level) and 57–78 (1 per cent confidence-level).

The hypothesis of a normal distribution of diploid numbers is in accordance with the facts. The modal class is 48, the limit values being 17 and 78. The larger the number of species studied in any systematic group, the more probable such a normal distribution appears. This is shown particularly well by a graph with the ordinate divided according to the Gauss integral, and the abscissa graduated with the diploid numbers, 17–78 (Fig. 1). The χ^2 test is not significant against the hypothesis, nor is the deficiency that may be observed for certain classes. The fact that there are fewer species with more than 48 chromosomes than with less (86 as against 122 for the eutherian sample,

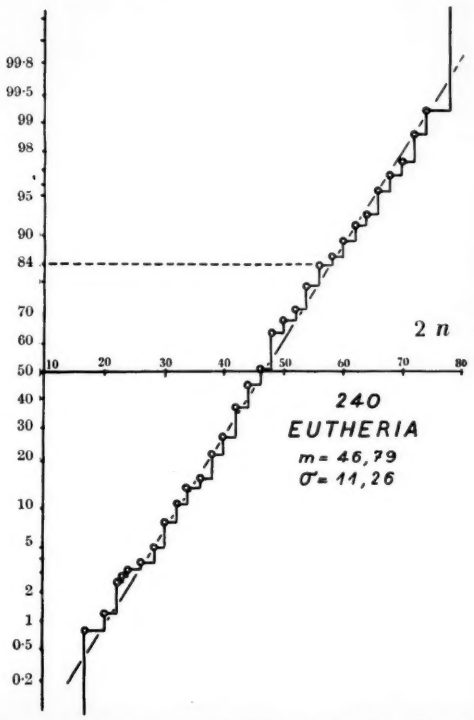

Fig. 1. Gaussian integral (ordinate) plotted against diploid number, $2n$ (abscissa) for eutherian mammals

64 as against 49 for the Muridæ sample) can easily be accounted for.

A variety of arguments make it probable that eutherians have developed from a pool of species with 40–56 chromosomes. The modal number 48 should be considered as primitive.

Normal distribution is characteristic not only of the eutherian sub-class as a whole, but also of the family of the Muridæ; it also appears in several sub-families. Lastly the spreading of diploid numbers in certain genera is almost as wide as that prevailing in the sub-class:

Limit values of the diploid numbers:	
sub-class (Eutherians)	17–78
family (Muridæ)	17–78
sub-family (Murinæ)	28–78
(Microtinæ)	17–62
(Gerbillinæ)	30–72
genus (*Microtus*)	17–62

It should be noted that the larger the number of species being studied in the group under consideration the wider the spreading grows, and that the majority of the chromosomal formulæ, in any of the more actively studied systematic categories, are to be found within the modal zone, 40–56.

This leads us to an important conclusion: the normal distribution shown by the eutherian sample, considered in its entirety, is the result, not of adding groups, some being characterized by low diploid numbers, others by high ones, the majority by modal or near-modal numbers, but of superimposing a series of normal distributions of modes equal or close to 48. This of course does not exclude the possibility of genera whose species, so far as they have been examined, are chromosomally identical (*Felis*) or very like one another (*Apodemus*). But in these cases one is dealing with systematic units which have been but little studied so far and a surprise remains possible, as is shown by *Bubalus* ($2n = 48$) and its contrast with other Bovidæ the diploid numbers of which are higher. The fact that there is a normal distribution is completely contrary to the often-expressed notion that a chromosomal evolution towards lower numbers is more likely than one towards higher. Both types of evolution are equally possible, one implying a loss, the other an increase of centromeres. On the other hand one must admit that an alteration of the chromosomal formula is all the less probable that it implies a more definite deviation from the mode. For it is difficult to imagine a strong deviation being realized at a single step; it must necessarily result from a series of transformations which take place successively and at intervals long enough for the delicate genic balance to be restored every time. The probability that each of these alterations does occur is so low that their accumulation, which is necessary to realize extreme values, can only be a very rare occurence.

Moreover, it is very strange that the great mutations in chromosomal structure represented by centric fusions (or the reciprocal fragmentation of one metacentric into two acrocentrics), as well as pericentric inversions, remain compatible with perfect stability of the genotype. Such big mutations are practically without effect; this is also proved by certain experiments of hybridization which bring out the competence of a spermatozoal genom of 12 chromosomes (*Microtus montanus*) with an ovule genom of 27 (*M. californicus*). Such chromosomal mutations are therefore secondary, later, that is, than the differentiation of new genera which can only have originated in species situated within the modal zone of an antecedent genus. A parallelism between morphological evolution and chromosomal evolution can only occur sporadically, according to my observations of Microtinæ, where the successive engagement of the great acrocentrics in the metacentric fraction of the genom is characteristic of the most advanced forms, and, from the chromosomal standpoint, ends in evolutionary blind alleys.

For the taxonomist, the chromosomal criterion, though of a peculiar nature since the chromosomal formula escapes the external pressure of selection and is only subject to internal conditions of cellular mechanics, is in general of no greater value than any other criterion. Its practical importance appears in connexion with certain 'difficult' groups in which species, otherwise very much alike, can be characterized by diploid numbers often very different (*Pitymys*, *Meriones*, *Gerbillus*). On the other hand, since apart from *Sorex araneus* no case of intraspecific chromosomal polymorphism in mammals is known, it can be assumed that a difference in the number of chromosomes implies a specific difference between two forms considered as sub-species by taxonomists. The cytologist can play the part of an umpire in the frequent controversies between the taxonomists who wish to increase and those who wish to decrease the number of species. He can also judge the value of certain sub-generic divisions. Furthermore, the chromosomal criterion makes a decision possible when the probability of a parallel chromosomal evolution between species with strongly aberrant chromosomal formulæ is negligible: thus *Microtus oregoni* must be placed close to *Ellobius lutescens*.

In conclusion, it is surprising that the facts observed in mammals should lead us to deny the evolutionary value of the greater chromosomal mutations and to preserve the notion of genic mutation as only a primary factor of evolution; certain authors throw doubt on the generally accepted separation between the two processes and it is indisputable that in such well known organisms as *Drosophila*, very small chromosomal mutations can be accompanied by important phenotypic effects.

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Buoyancy of the Cuttlefish

It is generally recognized that the cuttlebone not only serves the cuttlefish, *Sepia officinalis* (L.), as a skeleton but also gives it buoyancy. The cuttlebone contains gas spaces which give it a density of about 0·6. It is also known that cuttlefish kept in aquaria sometimes tend to float at the surface and appear to be lighter than sea water. Our experiments show that cuttlefish do change in density, and the difference between a 'light' and a 'heavy' cuttlefish is almost entirely due to a difference between the densities of their cuttlebones, the density and volume of the rest of the animal remaining almost constant.

The cuttlebone's structure is well described by Appellöf[1]. In a large adult it consists of about one hundred lamellæ of calcified chitin placed one above the other and held about two-thirds of a millimetre apart by vertical pillars. The spaces between these lamellæ form independent chambers which are themselves divided by about six thin membranes running parallel to the lamellæ. These chambers are sealed, laterally and anteriorly, by the very thick calcified layer forming the curved dorsal surface of the cuttlebone. Their posterior margins are formed by the

turned-over ends of the lamellæ; through these liquid can readily be extracted when a freshly dissected cuttlebone is placed under reduced pressure. The yellowish membrane, which is closely applied to this region, not only has a copious blood supply but also contains numerous ampullæ close to the bone which are connected by small ducts to the veins. In the histological sections the ampullæ and ducts contain no blood although blood can be seen in blood vessels which are smaller than these. It therefore seems very likely that exchanges of liquid occur here in the living animal.

Cuttlefish which had been kept in the aquarium for a week or two exhibited the wide range of cuttlebone densities 0·48–0·71 (mean = 0·60, *S.D.* = 0·06, $N = 38$) whereas those freshly caught and studied about R.V. *Sarsia* had cuttlebones the densities of which fell within the much narrower range of 0·57–0·64 (mean = 0·62, *S.D.* = 0·02, $N = 17$). The cuttlebones differ, not in the weight of dry matter per unit volume which is always close to 38 per cent, but in the amount of liquid which they contain. Thus a cuttlebone of density 0·7 contains about 30 per cent liquid, whereas a cuttlebone of density 0·5 contains about 10 per cent liquid. The remainder of the cuttlebone contains gas; but this gas is always at less than one atmosphere pressure for when a fresh cuttlebone is punctured under sea water this enters very rapidly and the density of the cuttlebone increases. This uptake of sea water cannot come from the solution of a very soluble gas, for when a fresh cuttlebone is gently dried and punctured out of water, air enters very rapidly. Within the duration of our experiments, the mass of gas per unit volume of bone remained almost constant whatever the density of the cuttlebone, so that in a bone of reduced density the pressure of the gas is even lower than usual. There can be no question of an evolution of gas raising the pressure and expelling liquid. The gas within the cuttlebone therefore plays a completely passive part in the density changes.

The pressure of gas in the cuttlebone is in no way balanced against the external hydrostatic pressure as in the swim-bladder of a fish. The cuttlebone is not an impermeable structure and some other mechanism must therefore be present both to make the gas space initially, and to prevent liquid entering under the high hydrostatic pressures found where these animals live. We have taken liquid—this is principally a solution of sodium chloride—from the cuttlebone and found that it has a higher freezing point than either sea water or cuttlefish plasma (sea water and cephalpod plasma are very close to one another in total osmotic concentration, Robertson[2,3]). For example, in animals caught in about 70 m., where the maximum possible hydrostatic pressure pushing water into the cuttlebone would be about 7 atmospheres, the liquid extracted from the cuttlebone had about three-quarters of the concentration of salts found in sea water. This suggests that simple osmotic forces play a major part in keeping water out of the cuttlebone for between this liquid and cuttlefish blood there would be an osmotic effect balancing about 5 atmospheres of pressure.

On coming towards the surface the equilibrium between osmotic effect and hydrostatic pressure could be simply restored by the movement of a little water from the bone with the consequent concentration of the solution in the bone. This would make the bone a little less dense and the animal slightly more buoyant. This cannot be the whole story because in many animals kept in the aquarium the density of the bone remains high, whilst in others (in which the buoyancy mechanism is disturbed) the cuttlebones become far lighter (densities about 0·5) than this explanation would demand. In the latter the loss of liquid from the bone is greater than that needed simply to establish a new balance between osmotic effect and the reduced hydrostatic pressure. Moreover the liquid readily available for exchange seems to have completely gone from such a bone for it is impossible to extract any liquid even under very reduced pressure. Since water and salts have left the cuttlebone against both the hydrostatic gradient and the salt gradient there must exist a mechanism capable of pumping salts out of the bone.

Thus the cuttlefish possesses in the cuttlebone a buoyancy regulating mechanism of a very ingenious kind. It is true that is more bulky than the swim-bladder, it occupies about 9 per cent of the cuttlefish's volume whilst a swim-bladder occupies only 5 per cent of a marine fish; but it also serves as a skeleton. It is almost free from the main disadvantage of a swim-bladder in that its upthrust will change only slightly on quick change of depth. Its useful depth-range is probably limited, for even were the liquid within the cuttlebone to be pure water, the osmotic effect could only balance the hydrostatic pressure of about 240 m. of sea water. Although the osmotic effect could prevent water entering the gas spaces, the cuttlebone had nevertheless to withstand the full hydrostatic pressure of the water above the cuttlefish. It is very strong, and even when dissected from the animal and tested in a pressure tank it only implodes at pressures corresponding to depths appreciably greater than those at which the animals live.

We are grateful to Mr. Dobson of the National Institute of Oceanography who tested for us the behaviour of the cuttlebone under pressure.

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Age of *Carcharodon megalodon*?

Maurice Lariche[1] states that the teeth of the gigantic extinct shark, *Carcharodon megalodon*, found on the bottom of modern oceans belong to the Pleistocene period. The report of the Challenger Expedition[2] states that some shark teeth from the same station were covered with a layer of manganese dioxide—one slightly, another deeply. Among them at Station 281, depth 2,385 fathoms, South Pacific, were found teeth of *Carcharodon megalodon*. Knowing even approximately the thickness and rate of growth of the manganese dioxide layer, we should be able to establish the geological age of embedded teeth much more exactly than before. According to Hans Pettersson[3] the growth-rate is 0·15 mm.–1·4 mm. every 1,000 years and on the upper surface of manganese dioxide nodules it is about 1½ times greater than on the lower.

The material for this investigation consisted of two teeth of *Carcharodon megalodon* from the above-mentioned Station 281. On the tooth *N*1 (Fig. 1) the manganese dioxide layer had almost completely disappeared, on the tooth *N*2 (Fig. 2) it covered quite a large area.

The thickness of the manganese dioxide covering was measured by a Swift petrological microscope. In the dental cavity, as usual for deep-sea deposits,

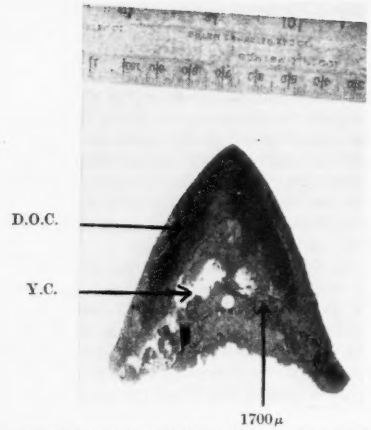

Fig. 1. Tooth *N*1, half natural size. D.O.C., deep olive colour; Y.C., yellow colour; 1700μ, maximal thickness of manganese covering.

dentine disappeared and the manganese dioxide layer was much thicker than on the surface—up to 1 cm. But the results of Pettersson's work are scarcely applicable to this part of the manganese dioxide layer: he investigated the surfaces of nodules, and the rate of formation of the manganese dioxide layer inside the deposits cavity is a separate problem to be solved by special investigation. To determine the age of the teeth, we take the lowest rate of nodule formation and the greatest depth of covering only.

The colour of the main part of the front side of tooth *N*1 resembled the light-coloured yolk of a hen's egg varying from barite—yellow to buff—yellow, or apricot yellow and pale orange according to Ridgeway[4]. It is seen clearly on a photograph as a light field surrounded from the top and sides by a dark stripe and spots, deep olive in colour, which is characteristic of fossil shark teeth found in the ground and from the ocean bottom deeply embedded in manganese dioxide. Yellow seems to be a natural colour and indirectly shows that this tooth is geologically young. The manganese dioxide layer was preserved in several small spots only, the thickest of which is 1,700 μ. Using the minimal Pettersson's rate—0·15 mm. in 1,000 years—it will require 11,333 years for deposition.

Tooth *N*2 is much darker than the first one, the deep olive colour of the fossil predominating, the yellow (cream buff) colour occupying a noticeably smaller surface, which on the front side formed rather a small triangle in the middle. The maximal thickness of the manganese dioxide covering is 3,640 μ. The minimal rate gives an age of 24,206 years. This means that *Carcharodon megalodon* became extinct in the latest Pleistocene or even survived until the Holocene period. To prove this, let us imagine that *Carcharodon megalodon* was really extinct in the middle Pleistocene—about 500,000 years ago. Then even the minimal rate shows that its tooth must be covered with a manganese dioxide layer about 75 mm. in thickness. Or let us imagine that the tooth in 500,000 years was covered with a coating of 4 mm. only. Then the rate of manganese nodules growth will be literally microscopic, that is, 8 μ in 1,000 years. This small, quite speculative figure sharply contradicts all the results of Pettersson's experimental work.

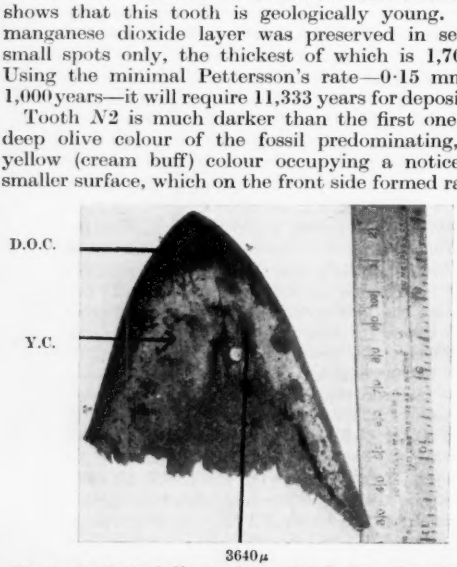

Fig. 2. Tooth *N*2, half natural size. D.O.C., deep olive colour; Y.C., yellow colour; 3640μ, maximal thickness of manganese covering.

Recognizing the relation between the age of the fossil and the thickness of the manganese dioxide covering, we can also determine the rate of nodule growth on the basis of the age of enclosed fossil. Up to 40,000 years, the carbon—14 method gives very exact results; but it needs a rather large quantity of organic material. This method is thence more suitable for such big deposits as whale earbones, covered with a manganese dioxide layer in the same manner as a shark tooth.

Knowing the age of the bone, we could estimate the overall rate of growth of the surroundings at different points and particularly in the deposit cavity.

I am grateful to Dr. J. D. H. Wiseman of the British Museum and Prof. J. E. Smith, head of the Department of Zoology, Queen Mary College, for their help and advice during this work and to Mr. S. V. N. Casey for the photographs.

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Distribution of Sodium in Compact Bone, as Revealed by Autoradiography of Neutron-Activated Sections

Recent work has emphasized the heterogeneous composition of compact bone. According to X-ray absorption, young osteons contain less calcium than the others[1]. Histological methods indicate that the mucopolysaccharides of the ground-substance are different in the preosseous layers, in the incompletely calcified osteons, and in the fully mineralized structures[2].

The distribution of sodium in compact bone was investigated in the same respect. Since histochemical methods are deficient in sodium, an attempt was made to identify this element after its activation in a nuclear reactor.

Pieces of ribs or tibiæ were taken from humans during surgical operations. They were kept in 96 per cent ethanol. It is obvious that part of the sodium, not firmly attached to the tissue, escaped in the fluid. Bones were cut in transverse sections with a saw, and these were ground down and polished by hand with emery paper.

The sections were submitted for six hours to a flux of $3{\cdot}5 \times 10^{11}$ neutrons/cm.^{2}/sec. in *Trico* (Triga Reactor supplied by General Atomic to the Government of Belgian Congo). It has been demonstrated[3]

that, after 3 hr., nearly all the radioactivity induced in bone originates from sodium-24. R. Loos, of the Department of Physics, confirmed this fact for our material by γ-spectrography.

After this delay, the sections were sandwiched between two 'Maximum Resolution' plates (Kodak Ltd.). When exposed for about 40 hr., the plates were developed in *D*-178.

Microradiograms of the sections were then obtained in 10 min. by exposure at a distance of 25 mm. from the tube of a Philips apparatus set at 5 kV. and 1·8 m.amp.

Fig. 1 shows an autoradiogram (*A*) and a microradiogram (*B*) of the same region of a human rib in transverse section. The darkening of the autoradiogram is not uniform. On the left, a large area which is not radioactive corresponds to an absorption cavity. Smaller white spots on the autoradiogram indicate Haversian canals as seen on the microradiogram. Besides these empty spaces which could be expected to be devoid of sodium, one may observe that the osseous substance itself is not uniformly radioactive. More precisely, the osteons not yet fully calcified, and thus appearing gray on the microradiogram, have given a weaker imprint on the autoradiogram. No doubt they are poorer in sodium than the completely calcified tissue.

Some sections were decalcified in ethylenediaminetetracetic acid; others were thus treated[4] before neutron activation. The former produce much paler images on the autoradiographic emulsion; the latter give the same pictures as untreated bone. It is thus confirmed that most of the sodium is linked in the mineral portion of bone tissue[5].

These observations show that the load of sodium, at least of sodium remaining in ground sections of bone fixed in alcohol, parallels the load of calcium as indicated by X-rays. The concentration of sodium is lower in young osteons than in old ones, just as it is lower in the skeleton of young rats than in that of old rats[6]. It seems that an osteon reaches saturation nearly at the same time for both calcium and for sodium.

I wish to thank the Commission Consultative des Sciences Nucléaires du Congo Belge et du Ruanda-Urundi for financial support and use of the reactor *Trico*, Mr. E. M. de Dorlodot, director of the reactor, for kind collaboration, and Mr. L. Mandiangu for technical assistance.

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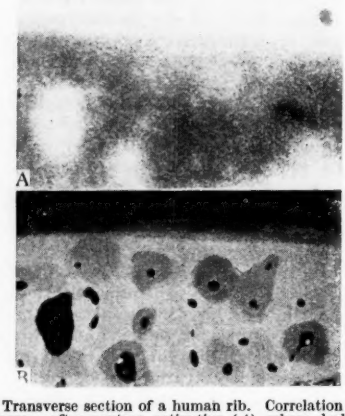

Fig. 1. Transverse section of a human rib. Correlation between autoradiogram after neutron activation (*A*), where darkening is related to sodium concentration, and microradiogram (*B*), where white areas indicate the greatest amounts of calcium. The absorption cavity (on the left) and the Haversian canals are not radioactive. The less calcified osteons contain less sodium than the others. (×*c.* 25)

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Dimorphism and Size Distribution in *Velella* and *Physalia*

WOODCOCK[1] attributed right- and left-handedness in *Physalia*, the Portuguese man-of-war, to a selective advantage in avoiding entrapment in windrows of *Sargassum* weed and floating debris. This selective advantage was presumed to be due to differences in sailing patterns through convection cells in the surface water of the northern and southern hemispheres. The absence of *Sargassum* from the South Atlantic and the paucity of debris in the barren, blue waters in which *Physalia* is characteristically found would seem to invalidate this hypothesis. On the other hand Woodcock's arguments concerning food concentration by the convection cells and sailing patterns are more convincing. Woodcock[2] continued to favour the hypothesis that there are significant differences in abundance of the two forms between the northern and southern hemispheres. Other authors[3,4] have commented on this problem; however, none has pointed out that the more extensive literature on *Velella*, long known to be dimorphic[5], shows no statistically reliable difference between the abundance of the two forms in the northern and southern hemispheres.

The fact that Agassiz[6] found only left-handed specimens present in more than two thousand *Velella* collected along the shores of the north-west Atlantic while Chun[7] found 71 left-handed and 6 right-handed *Velella* off Africa in the north-east Atlantic would indicate an east-west or zonal difference. Chun's[7] findings are confirmed by the results of Moser[8]. In a recent paper, Savilov[9] reported left and right-handed specimens of *Velella* from the north-west and south-west Pacific. Of more than 250 specimens examined by the author from the north-east Pacific all were left-handed. Thus, if there is an east-west difference in relative abundance of the two forms, the results available to date indicate that the situation in the Pacific is the reverse of that in the Atlantic.

Savilov advances a hypothesis that appears to solve this problem. In the northern hemisphere, left-handed specimens of *Velella* move to the left of the wind direction due to the anticyclonic wind circulation over the ocean. The left-handed specimens are therefore concentrated along the outer edges of the distribution. The right-handed *Velella* move to the right of the wind direction and are concentrated in the centre of the distribution. Thus one should find the left-handed specimens near shore. In the anticyclonic wind circulation of the southern hemisphere the left-handed *Velella* are concentrated in the centre of the distribution with the right-handed specimens more abundant along the borders of the distribution. The only results which weaken this argument are the exclusively left-handed specimens taken by me off California. Many of these were collected more than 300 miles off shore.

Savilov[9] found large specimens of *Velella* most abundant in the region of 40° N. lat. in the Kuroshio Extension. Young and larval forms were common in the south and far western parts of the Pacific. He attributed this size distribution to the wind and current patterns. An alternative explanation follows:

My studies of *Velella* off California, extending over a period of six years (unpublished results), show a marked seasonal appearance of *Velella* at the surface. This is confirmed by a careful examination of the previously published literature. The post-larval specimens first appear at the surface in very late December or early January and continue to reach the surface through the spring. The largest specimens are found in late autumn and early winter.

Examination of the track of the *Vityaz* shows that the stations in the Kuroshio Extension were occupied from July to November when neither larval nor very young forms would be present. The southern and far-western stations were occupied in December, January, February, July and August when the largest specimens are rare if present at all. Larval and young specimens would be present in December, January and February. Thus the size distribution Savilov describes in general terms can be more easily explained by seasonal differences. The data he presents do not allow a more precise analysis of the problem. One might also expect to find mean length differences between local populations because of different sea surface temperatures. However, these would be much smaller than those due to seasonal appearance and growth.

From the above it is apparent that the origin and occurrence of the different sizes and morphological forms of *Velella* and *Physalia* are not yet satisfactorily explained. Of the several variables that appear to be involved, seasonal appearance and growth have not been properly considered in previous reports.

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Penetration of the Liver-fluke, *Fasciola hepatica* into the Snail, *Limnaea truncatula*

The life-history of *Fasciola hepatica* has been recounted in nearly every text-book of zoology or of parasitology since it was elucidated by R. Leuckart (1881–82) and A. P. Thomas (1881–83). In spite of this and much original work by other investigators our knowledge of (*a*) the form which penetrates the snail host and (*b*) the manner of its penetration is misconceived. For example, in the modern account given by G. Lapage[1] it is stated that once a snail has been found, the miracidium "applies the papilla at its broadest, anterior end to the soft skin of the snail and, spinning by means of its cilia on its long axis, it drives the papilla into the snail and penetrates the snail's body". Other writers have introduced something between this sort of statement and the more correct idea, succinctly expressed by Faust[2], that penetration "is accomplished by the secretion of digestive enzymes elaborated in the so-called 'penetration glands' which discharge the secretion at the anterior end of the miracidium". It is difficult to prove that 'digestive secretions' are produced, or even to demonstrate the cytological effects produced by a penetrating larva which is smaller than some ciliated protozoa. It is here shown, for the first time by means of photomicrographs, that the miracidium creates a perforation in the snail's integument by the loosening, cytolysis and abstraction of epithelial cells, an action which appears to be chemical rather than mechanical and is probably the result of enzyme activity. It will be shown also that, because the miracidium loses its ciliated epithelium and is in other ways transformed before penetration is effected, it is an early sporocyst and not a miracidium which enters the snail.

The miracidia of *Fasciola hepatica* are not as efficient in locating and penetrating snail hosts as some studies of their tropistic behaviour lead us to suppose. In the immediate vicinity of a snail many larvæ swim to and fro without ever attacking, and many more encounter the snail but do not succeed in adhering to it, much less penetrating it. When contact is established, however, the miracidium butts the snail several times, and it is this action which has given the false impression that the rotating larva is boring like an auger into the snail when in fact is is merely trying to attach itself. Early adhesion is so light that no matter how carefully snails are fixed and prepared for sectioning, the larvae fall away. Attempts to adhere often fail and the larvæ swim away to try again elsewhere. After several unsuccessful attempts of this kind miracidia seem to be exhausted; their swimming movements become erratic and eventually they die. Some such moribund larvæ lose their ciliated epithelium, however, and undergo partial metamorphosis into ovoid young sporocysts. According to Mattes[3], the anterior and posterior 'Klebdrüsen' are concerned with adhesion. Careful study has not so far revealed these paired unicellular glands, although their large nuclei and their position beneath the first- and second-tier epithelial cells of the miracidium should make them conspicuous. Early attachment is more probably brought about by suctorial action of the anterior papilla which, by its introversion, presses the first-tier epithelial cells hard against the snail's integument, mucus assisting adhesion. Once attachment is established, retraction of the papilla would create a saucer-like space between the anterior nonciliated pit of the larva and the snail's integument, and this would serve for the reception of secretions of the gut and the unicellular pharyngeal glands. Not until marked cytolytic effects have been produced in the epitheium of the snail does the anterior papilla of the miracidium penetrate into this layer (Fig. 1, *A*1, *A*2).

Complete penetration of the larva into the snail takes only about 30 minutes from the time of adhesion. During this period the larva is a sac-like object which occasionally contracts and relaxes but which certainly does not rotate. At the end of the period, when failure to penetrate seems likely, the larva suddenly disappears into the snail. Sections indicate that about the middle of the period the anterior papilla is only slightly extended and is approaching the sub-epithelial tissues of the snail, amidst the debris of loosened and cytolysed epithelial cells (Fig. 1, *A*2).

As the larva presses into the cytolysing mass, some of these cells are heaped externally at the margin of the opening (Fig. 1, *A*1). At the same time, larval epithelial cells are becoming detached, although

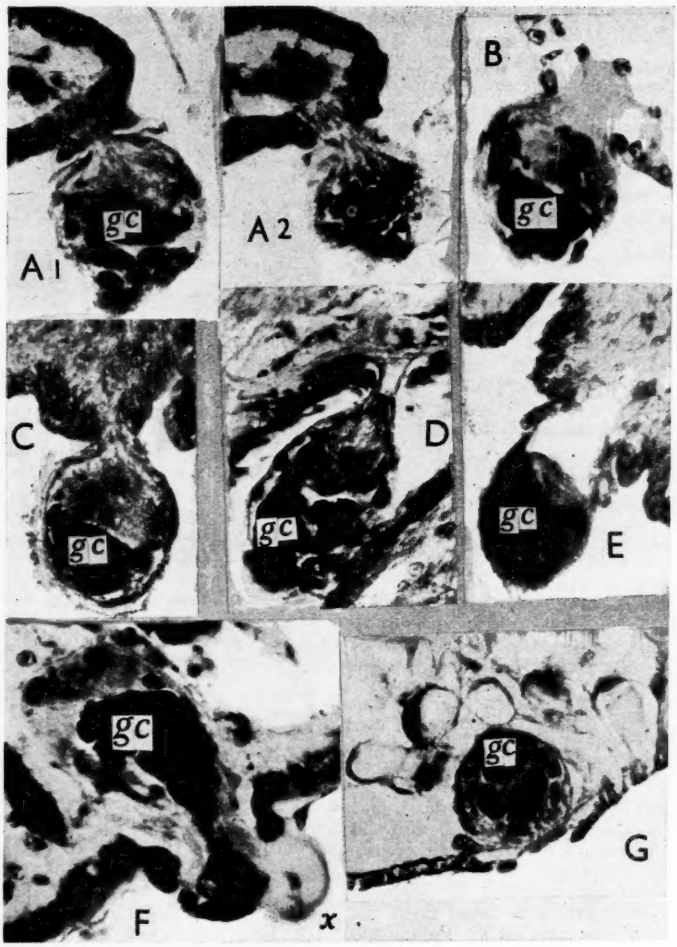

Fig. 1 *A-E*, attached, metamorphosing miracidia; *F*, penetrating sporocyst; *G*, entered sporocyst. *A*1 and *A*2 are adjacent sections. *gc*, germinal cells. (Various magnifications; *D* and *F*, oil immersion, the remainder $\frac{1}{6}$ in. objective)

the anterior ends of the first tier cells are trapped in the raised margin of the opening. Cytolysis is very evident when attachment is to the mantle, which has a shallow epithelium, nuclei standing out starkly (Fig. 1, *B*), and the papilla is seen to be retracted in suctorial action.

The papilla is now becoming attached to the sub-epithelial layer of the snail (Fig. 1, *C* and *D*) and damage to this tissue soon becomes evident. As the larva draws its anterior end further into the cytolysing host cells it presses aside the damaged epithelium forming the rim of the opening, which is thus enlarged. The larva then contracts momentarily inside a kind of sac formed partly of cytolysing cells and partly of its own discarded epithelium, together with some mucus (Fig. 1, *E*). This stage immediately precedes the swift final thrust which takes the extending larva into the body of the snail. Available sections show the thrust in several phases, the larva becoming constricted at different levels along the antero-posterior axis progressively as it squeezes through the opening. Only one stage is shown here (Fig. 1, *F*) and in this, the cytolysed sub-epithelial tissue (*x*) is being pressed out through the opening as the larva enters the snail. As the larva completes its entry, the damaged cells at the rim of the opening are drawn inwardly, partially sealing the opening (Fig. 1, *G*). The posterior end of the larva is seen as a zone of dense tissue produced by intense contraction.

The larva which enters the snail is certainly not a miracidium, although it retains the eyes, the gut and other organs, and also the germinal cells; it is a young sporocyst covered by what was formerly sub-epithelial tissue, carrying with it into the snail some epithelial and other debris. The miracidium may be regarded as a form which serves to implant the sporocyst in the body of the snail host by what appears to be an elaborated process of external digestion. A fuller account of this work, with more adequate discussion, will be published soon. My thanks are due to Mr. A. T. Green, for making the photographs.

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Production of Seedless Hops by Interspecific Pollination

THE difficulty in inducing hop cone formation through the application of growth-stimulating substances has been well illustrated by Seeley and Wain[1] who found that, at best, it was possible to achive only temporary stimulation of cone growth in experiments using some 22 growth-stimulating substances, as well as pollen extracts.

In order to test the stimulation produced by foreign pollen, a small-scale experiment was conducted using the cultivated variety Late Clusters (of the species *Humulus lupulus L.*) and crossing this with the wild hop, *Humulus japonicus* Sieb. and Zucc. These two species, *H. lupulus* and *H. japonicus*, are very distinct. The former is a perennial with a chromosome number[2,3] of 20 and the latter an annual of 17 chromosomes in the male, 16 in the female[4].

Table 1 contains measurements, based upon 30 samples, from unpollinated and pollinated cones.

Table 1

	Average length (mm.) of bracts	Average length (mm.) of bracteoles	Average length (mm.) of internodes	Average No. of nodes
Pollinated with *H. japonicus* pollen	12·9	14·7	1·5	8·4
Unpollinated	10·1	11·3$_2$	1·0	11·1

The stimulation of bracts, bracteoles and internodal length in the cone of Late Clusters produced by pollen of *H. japonicus* is very noticeable, though it is unquestionably less than that produced by pollen from the male plants of Late Clusters. Fig. 1 shows pollinated (*H. japonicus* pollen) and unpollinated cones collected at the same stage of maturity.

Early workers, including Salmon and Amos[5], had shown the importance of pollination (by pollen of the same species) upon increasing yield. Their results indicated, as in this experiment, that pollination decreased the number of nodes formed, though they did not discuss this effect which is based upon the indeterminate growth of the cone apex. Pollination brings this apical growth to a halt. The length of time during which such growth continues without pollination varies with variety, being longer in a variety such as Late Clusters than Fuggles. These facts help to explain the difference among varieties in the influence of pollination upon yield. In some varieties the smaller size of bracts and bracteoles in unpollinated cones is offset by a continued growth of the apex, and a resulting increase in their number. Conversely, in those varieties in which apical growth ceases early, pollination is vital for the stimulation it produces upon bract and bracteole size.

As with normal pollination, the effect of interspecific pollination is to reduce greatly the critical 'burr' stage, when the cone is so susceptible to disease.

Following interspecific pollination, the ovary is stimulated at the same time as the internodes, bracts, and bracteoles. However, since fertilization does not occur, due to the difference in chromosome number between the species, no embryo, endosperm or seed develops. The matured pistil, much smaller than the normal product of pollination, remains empty.

It is not possible to draw conclusions regarding the commercial usefulness of such interspecific pollination in hop cultivation. Extensive experiments would be necessary to determine the extent to which such pollen will effectively carry by wind, the practicability of

Fig. 1. Cones pollinated with pollen from *H. japonicus* on left; unpollinated cones of late clusters on right. Scale in mm.

growing the weed hop in cultivated fields and the desirability of the cone, formed without seeds but containing abortive pistils. This does, however, illustrate a principle which may prove of some importance, namely, that stimulation may be produced in hops by pollination which cannot lead to fertilization, and consequently, seed formation. Moreover, it suggests that the triggering mechanism for stimulation of cone growth in hops must occur at the time of pollination, with fertilization providing only a secondary boost, if any boost at all.

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Vein Anastomoses in the Leaves of Long Shoots of *Ginkgo biloba*

IT has recently been found that four types of vein unions occur in the leaves of *Ginkgo biloba* L. and that long shoots have a significantly higher average per cent of leaves with anastomoses than short shoots[1]. In the present examination of 2,249 leaves collected from 154 long shoots from 16 trees it was found that the leaves from the median portion of the long shoots have a higher per cent of anastomoses than either the basal or apical leaves. It was also found that considerable variation existed in the percentage of leaves with anastomoses in the samples taken from various trees. An average of 33 per cent of the 2,249 leaves had one or more anastomoses; but the range in the individual 16 trees extended from a low of 7·3 per cent to a high of 71·2 per cent.

By collecting the leaves in a manner so that the position of each leaf on its shoot was known, the percentage of leaves having anastomoses could be determined for each leaf position. Because of multiple anastomoses occurring in many leaves, the total number of anastomoses at each leaf position is best expressed as an anastomosis index, which is derived by dividing the total number of anastomoses by the total number of leaves at a given leaf position. In Fig. 1 results are presented which show the relationship between anastomosis index and leaf position. The median leaf positions (10–13) show an anastomosis index more than double that of the most basal (1–5) and the most apical (19–20) leaf positions.

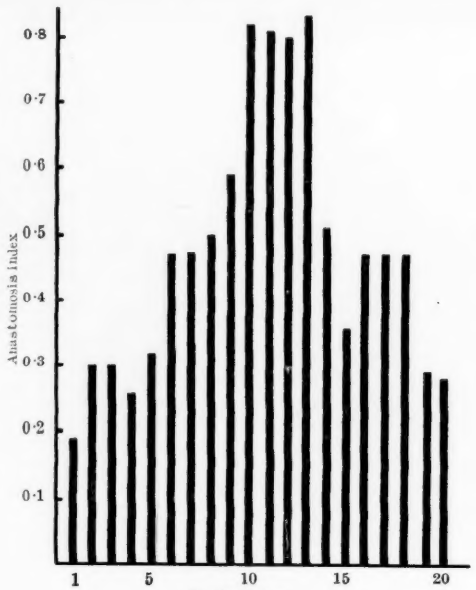

Fig. 1

The number of leaves studied at each leaf position averaged 112 and varied from 154 at leaf position 9 to 18 at leaf position 20. The first 13 leaf positions were represented by a minimum of 121 leaves each, while those from 14–20 were represented by an average of 41 leaves. The number of leaves from the apical leaf positions is lower because many of the long shoots studied had less than 15 leaves. Leaves above leaf position 20, which were few in number, were not included in this study.

While surveying the literature on *Ginkgo* some striking similarities in the pattern of auxin distribution and that of anastomosis frequency were noted. The concinnity of these distribution patterns can be elucidated by the hypothesis that the percentage of anastomoses in the leaves of long shoots of *Ginkgo biloba* is in some way correlated with the amount of auxin present in the shoot at the time of leaf differentiation. The similarity of the pattern of auxin production and anastomosis distribution in the long shoots of *Ginkgo biloba* can be seen by comparing the data results above with those of the auxin diffusion experiments of Gunckel and Thimann[2]. They found that in very young long shoots, "the peak of auxin production lies in the middle or toward the base of the shoot".

Foster[3], and Gunckel and Wetmore[4] report that there is no fundamental difference in the organization of the apical meristems of long and short shoots. Gunckel and Wetmore[4] report that the early stages of growth and differentiation in long and short shoots are indistinguishable. Gunckel and Thimann[2] compared the amounts of diffusible auxin in 'putative' long and short shoots during early ontogenetic development. They found that in both shoot categories the amount of auxin increased to a peak and then decreased during the early stages of bud expansion. In short shoots auxin production continues to diminish, however, in long shoots auxin production undergoes a further, and much higher, increase as the shoot begins to elongate. Results collected during this work show that in leaf positions 1–5 the average anastomosis index is 0·28 whereas in leaf positions 6–18 the anastomosis index averages 0·61. A tentative explanation for this anastomosis distribution is that approximately the first five leaves of a long shoot are produced under a 'short shoot regime', a regime which is apparently exactly the same as that of a short shoot both in morphological differentiation and in auxin production. The more median leaves of long shoots, however, are differentiated under a 'long shoot regime' during which auxin production is higher and the shoot is elongating. The higher anastomosis index of these leaves is in harmony with the data on auxin diffusibility in long shoots given by Gunckel and Thimann[2], and again tends to support a correlation between anastomosis frequency and auxin.

At present no experimental evidence is available which tests a hypothesis linking auxin concentration and anastomosis frequency in *Ginkgo*; however, experiments which may shed some light on this relationship are now under way.

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ENTOMOLOGY

Pentachlorocyclohexene as a possible Intermediate Metabolite of Benzene Hexachloride in Houseflies

Sternberg and Kearns[1] have reported that both γ-benzene hexachloride (γ-BHC)-resistant and susceptible houseflies can dehydrochlorinate γ-BHC to pentachlorocyclohexene (PCCH). Their evidence relied on the formation of 1-chloro-2:4-dinitrobenzene when the method of Schecter and Hornstein[2] for the determination of γ-BHC was applied to flies treated with the insecticide. This method involved the reduction with zinc and acetic acid of unchanged γ-BHC and PCCH to benzene and chlorobenzene followed by nitration to form *m*-dinitrobenzene and 1-chloro-2:4-dinitrobenzene respectively. Bradbury and Standen[3], working with a different strain of resistant and susceptible houseflies, used an isotopic dilution technique to determine any γ-PCCH present after γ-BHC treatment but failed to find the large amounts reported by Sternberg and Kearns.

Work has been carried out at this laboratory on a strain of dieldrin-resistant (*R*-strain) and susceptible *Musca domestica* obtained originally as pupæ through the kindness of Dr. J. R. Busvine. The dieldrin-resistance of the *R*-strain has been built up to a high level by exposure of the larvæ to dieldrin alone. The same insects have, however, become highly resistant to γ-BHC. Studies of the resistance mechanism have been made using the α-, γ- and δ-isomers labelled with carbon-14. Preliminary experiments were made using the technique of Sternberg and Kearns. Flies were treated with 2 μgm. of the radioactive BHC isomers in acetone solution, 2μl. of the solution being applied topically to the dorsal thorax of each fly. After 3 hr. at 25°C. the flies were ground under acetic acid and subjected to the Schecter-Hornstein reduction and nitration procedure. The 1-chloro-2:4-dinitrobenzene was separated from the *m*-dinitrobenzene by chromatography on paper impregnated with castor oil[4] and when scanned to detect radioactivity[5] showed that similar amounts of

chlorodinitrobenzene occurred from the flies treated with any of the three isomers amounting to about 17 per cent of the adsorbed dose. Using his more specific isotopic dilution technique Dr. F. R. Bradbury kindly made some determinations of γ-PCCH in flies from the same strain which had been treated with γ-BHC-^{14}C as decribed above. He found only 1·5 per cent of the adsorbed dose present as γ-PCCH and suggested that a metabolite other than PCCH might be giving rise to chlorobenzene on reduction.

In view of this possibility, treated flies were homogenized under water and ether together with added non-radioactive γ-BHC and γ-PCCH carriers, centrifuged and the layers separated. The ether and water layers were taken almost to dryness and then reduced using the Schecter-Hornstein procedure. The nitrated reduction products were separated chromatographically. Only 2·3 per cent of the adsorbed dose was found as chlorodinitrobenzene from the ether layer, whereas 4·7 per cent was found from the water layer. Further, when flies treated with γ-BHC-^{14}C under the same conditions were ground up under acetic acid and subjected to the Schecter-Hornstein procedure but in the absence of zinc a further 2·4 per cent of the adsorbed dose was accounted for as chlorodinitrobenzene. Similar determinations on the ether and water layers in the absence of zinc yielded only trace amounts of chlorodinitrobenzene. This suggested that free chlorobenzene was occurring as a metabolite in the flies and this would almost certainly be lost when the ether extract was concentrated prior to the determination. Thus 9·4 per cent of the adsorbed dose was accounted for as chlorodinitrobenzene compared with 16·7 per cent determined when the reduction was carried out directly on the flies. This discrepancy may be due to losses occurring during the fractionation and concentration of the ether and water extracts but it may be that some other metabolite is present which would give rise to chlorobenzene on reduction but which is lost during fractionation and concentration.

These experiments suggest that the chlorodinitrobenzene obtained using the method of Sternberg and Kearns arises from at least three sources; chlorobenzene occurring as a trace metabolite, some water-soluble metabolite which under reducing conditions gives rise to chlorobenzene and PCCH recovered in the ether extract. Thus the values reported by Sternberg and Kearns for PCCH may be artificially high although a similar fractionation procedure would have to be carried out using their strain of flies before this could definitely be established.

In connexion with the presence of chlorobenzene in the treated flies it is of interest that small amounts can be detected together with trichloro- and dichlorobenzenes in the air drawn over flies treated with ^{14}C-α-BHC.

The fact that large amounts of PCCH cannot be demonstrated in BHC-treated flies does not imply that the main metabolic pathway is not through PCCH which may only be a transitory intermediate. However, when 5 μgm. of γ-PCCH in soya bean oil was injected into flies prior to treatment with ^{14}C-γ-BHC no build up of radioactive γ-PCCH could be detected after a 3-hr. period. This suggests that monodehydrochlorination is not the first step in any major pathway for the metabolism of γ-BHC by houseflies and lends support to the suggestion of Bradbury and Standen[6] that direct substitution of the chlorine atoms occurs. The detection of γ-PCCH together with other chlorinated benzenes may mean that an alternate pathway by dehydrochlorination is possible though of secondary importance.

These experiments are part of an investigation into the mechanisms of dieldrin resistance in Diptera, supported in part by a research grant from the World Health Organization.

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Insecticidal Action of Ethylene *Bis*dithiocarbamates

The acaricidal action of manganous ethylene *bis*dithiocarbamate ('Maneb') has been observed by Rich[1] and by Morgan, Anderson and Swales[2]. Rich noted that 'Zineb', the related zinc salt, did not kill any stage of the two-spotted mite, *Tetranychus bimaculatus* (L.). I have not been able to find any mention in the literature of insecticidal action of either 'Maneb' or 'Zineb'.

Preliminary experiments in the greenhouse on the control of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westw.) showed that sprays of wettable 'Maneb' and 'Zineb' are insecticidal to larvæ and adults of this insect. Subsequent experiments and observations carried out in a controlled-temperature, plant-growth room indicated that these fungicidal compounds probably have a toxic action different from most insecticides. 'Perthane' and 'Malathion', conventional insecticides which were also used in these experiments, killed the majority of the larvæ affected by them within three days and after seven days all such larvæ were desiccated and shrivelled. In contrast, larvæ affected by 'Maneb' or 'Zineb' usually did not die until one to three weeks after treatment. During this post-treatment period the affected larvæ did not moult; however, they grew to the extent of cuticular limitations and became bloated and globular. When they were teased off the leaf, movements of the styli could be detected. Larvæ that were not affected by 'Maneb' or 'Zineb' moulted similarly to those in check treatments and retained their normal appearance.

Table 1. Relative toxicities of 'Perthane', 'Maneb' and 'Zineb' to greenhouse whitefly

Material	Slope of regression line	*LD*50 grams of actual toxicant per litre of spray	5% fiducial limits	
50% wettable 'Perthane'	2·331±0·166	0·275	0·249	0·304
70% wettable 'Maneb'	1·761±0·161	0·338	0·241	0·471
65% wettable 'Zineb'	2·745±0·185	1·511	1·381	1·654

Table 1 shows the relative toxicities of sprays of 50 per cent wettable 'Perthane', 70 per cent wettable 'Maneb', and 65 per cent wettable 'Zineb' to a mixed population of first and second instar larvæ of *T. vaporariorum*.

Further work is being done to learn more about the mode of action of ethylene *bis*dithiocarbamates on insects.

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MICROBIOLOGY

Tocopherols in Micro-organisms

ALTHOUGH the tocopherols, and in particular α-tocopherol, are widely distributed in Nature, they have not so far been identified in micro-organisms. The only micro-organism that has been adequately studied is bakers' yeast, which has been found to contain no tocopherol, although it contains another substance with vitamin E-like activity[1]. In view of recent work connecting α-tocopherol with some fundamental cellular processes, such as the reduced diphosphopyridine nucleotide-cytochrome *c* reductase system[2], the regulation of succinate oxidation in rat liver mitochondria[3] and the synthesis of adenosine triphosphate[4], it was of interest to investigate the occurrence of tocopherols in a range of micro-organisms. The following organisms were grown in deep culture, using suitable media, the general composition of which is shown in Table 1: three strains of *Esch. coli.*, 8196 and two vitamin B_{12}-requiring mutants, N.I.R.D. and *M*200 (*C*181 strain); *Streptococcus fæcalis R.*; *Bacillus cereus* (strain 5); *Staphylococcus aureus* 4163; the nitrogen-fixing bacterium, *Rhizobium leguminosarum* 317; three sulphur-requiring, chlorophyll-containing bacteria, *Chromatium* 8379, *Chlorobium thiosulphatophilum* and *Thiopedia*; two chlorophyll-containing protozoa, *Ochromonas malhamensis* and *Euglena gracilis*; and one non-chlorophyll containing protozoan, *Tetrahymena pyriformis*.

After suitable incubation periods, when heavy growth was obtained, the micro-organisms were centrifuged off, washed and weighed. Quantities varying between 2 and 20 gm. were taken for analysis. Each batch of micro-organisms was extracted exhaustively with boiling ethanol, and the lipid fraction obtained by ether extraction after dilution with water. This was analysed for tocopherols by methods including two-dimensional paper chromatography previously described[5,6].

The results (Table 1) show (*a*) that α-tocopherol is the only tocopherol observed in these organisms, (*b*) that its presence is not restricted to any one type of organism, (*c*) that it is found only in organisms containing chlorophyll, although it was not found in all of them. The results support a previous suggestion[6] that tocopherol in plants might be formed from the same phytol precursor as chlorophyll. That there is a close relationship between tocopherol and chlorophyll synthesis is further borne out by the difference in the figures for α-tocopherol in *Ochromonas*, depending on whether this organism is grown in the light or the dark. Under dark conditions, *Ochromonas* develops very little green pigmentation and the corresponding tocopherol analysis is only about a sixth of that obtained from the same organism grown in daylight. Dam *et al.*[7,8] have considered synthesis of vitamin K to be linked with chlorophyll synthesis. It is perhaps of interest that *Chromatium*, which did not contain detectable amounts of tocopherol, was found to contain large amounts of vitamin K_2 and ubiquinone 50. The latter substances were identified by paper chromatography and ultra-violet absorption curves on the eluted fractions.

Table 1. TOCOPHEROLS IN MICRO-ORGANISMS

Organism	Medium	Tocopherols (μgm./gm. wet wt.) α	non-α
Esch. coli (3 strains)	Lactose-peptone broth.	ND	ND
Strept. fæcalis R	Buffered peptone yeast ext. glucose.	ND	ND
B. cereus (strain 5)	Peptone-Lemco.	ND	ND
Staph. aureus (4163)	" "	ND	ND
Rhizobium leguminosarum 317	Salts + mannitol + yeast ext.	ND	ND
Chromatium 8379	Synthetic + Na_2S	ND	ND
Chlorobium thiosulphatophilum.	Synthetic + Na_2S	1·7	ND
Thiopedia	Synthetic + Na_2S	ND	ND
Ochromonas malhamensis (grown in light)	'Vitamin B_{12} inoculum'	88·0	ND
Ochromonas malhamensis (grown in dark)	'Vitamin B_{12} inoculum'	14·2	ND
Euglena gracilis	Acetate-pepsin-yeast ext.	155	ND
Tetrahymena pyriformis	Protease-peptone-yeast ext. glucose.	ND	ND

ND, not detected.

We are indebted to Miss M. E. Adams and Mr. K. R. Butlin, of the Chemical Research Laboratory, Teddington, and Dr. J. Kleczkowska, of Rothamsted Experimental Station, for cultures of the sulphur-bacteria and *Rhizobium*, respectively, and for advice on the propagation of these organisms.

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Production of Colicines in Simmons's Citrate Agar

ANTIBIOTIC substances produced by strains of the family Enterobacteriaceæ are known as colicines[1,2]. A recent review by P. Fredericq[3] indicates that these substances are of particular biological interest: colicinogenic strains resemble in some aspects lysogenic strains, and colicines have certain characteristics similar to those of bacteriophages. Colicinogenic strains behave as pathogenic agents, which can be transduced from colicinogenic to non-colicinogenic strains by culture in common[3-5].

In some experiments on the transduction of colicinogeny from cultures producing colicine I to non-colicinogenic cultures, I observed that colicinogeny was transduced when cultures where plated together on nutrient agar (for details of technique see refs. 3–5), but not when plated on Simmons's citrate agar with glucose 1‰ w/v.

It was therefore thought that colicine I is not produced in Simmons's citrate agar, and a number of strains producing colicine I, as well as some other colicinogenic strains, were tested in this synthetic medium for production of colicine.

The colicinogenic strains were stabbed on plates of Difco Simmons's citrate agar with glucose (1‰ w/v), eight per plate, according to Fredericq's technique[3], and incubated at 37° for 48 hr. After sterilization with chloroform vapour, a sensitive indicator strain was seeded over the surface of the plates[3]. After 24 hr. incubation at 37°, around the cultures which produce colicines in the synthetic medium, clear zones of inhibition of the sensitive strain are produced.

Table 1 shows results for 73 strains; 61 of these strains were typed in our Laboratory and 9 are type cultures of the collection of P. Fredericq and produce

Table 1. Production of colicines by colicinogenic strains in Simmons's Citrate agar

Type of colicine produced	No. of colicinogenic strains	No. of strains producing colicine in Simmons's citrate agar
I	30	0
E	12	5
E+*I*	11	5
V	4	4
K	4	4
X	3	3
B	1	1
D	1	1
A	1	1
F	1	1
G	1	0
C	1	1
H	1	1
S_3+*I*	1	1
S_5	1	0
Total	73	28

colicines *B*, *D*, *A*, *F*, *G*, *C*, *H*, S_3+I and S_5; finally, a very strong, non-typed colicine, produced by the strain *Mutaflor* of Prof. Nissle, largely produced in Germany by the A. G. Hageda for the treatment of 'Dysbakterie'[6], is defined as colicine *X*.

Strains producing colicine *I*, a part of strains producing colicine *E*, or colicines $E+I$ simultaneously, and type cultures producing colicines *G*, *H*, and S_5 did not produce any inhibition zones on the strain sensitive to the indicator (Table 1).

Strains producing colicines *V* and *K*, some of the strains producing colicines *E* or $E+I$, and type cultures producing colicines *B*, *A*, *C* and S_3+I, gave smaller inhibition zones of the indicator in Simmons's citrate than in nutrient agar.

Finally, the type cultures producing colicines *D* and *F*, and the cultures producing colicine *X* gave very large inhibition zones in the synthetic medium.

This observation can be of practical value in the typing of the colicinogenic strains. This typing was found to be important in epidemiological studies of infantile diarrhœa due to *Escherichia coli*[7]. A large number of colicinogenic *E. coli* isolated from epidemics produce colicine *I*[8] or colicine *I* together with another colicine (unpublished results).

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Lysogeny in the Genus *Proteus*

A lysogenic strain of *Proteus* species was detected by Fejgin in 1924[1] but no systematic attempt has ever been made to ascertain the prevalence of such strains. We have investigated the incidence of lysogeny using 23 *Proteus* strains for which we have previously isolated lytic phages from sewage[2]; media used have been previously described[2,3].

The Fisk technique[4] using overnight broth cultures gave uniformly negative results, and other methods of induction were then used. The 3 methods used, details of which will be published elsewhere were: (1) individual cultures of the 23 strains were grown in broth for 10 days at 37°C.; (2) ultra-violet irradiation according to the method of Gots and Hunt[5]; (3) all possible combinations of pairs of the 23 strains were grown together in broth for 10 days according to the Scholtens' method[6].

Cultures thus obtained were centrifuged to clarity, kept at 56°C. for 45 min. to inactivate remaining bacteria, and then tested for phage activity by a modification of the agar layer technique[2]. Using 49 different *Proteus* strains as indicators, 12 of the 23 strains were found to be lysogenic. The three induction methods apparently possess a degree of species specificity, in that not all lysogenic strains were induced by all three methods, and the strains induced by method (3) were all *mirabilis* species, while 4 of 5 induced by method (2) were *vulgaris* species. In only one instance did a phage derived from a *vulgaris* act on a *mirabilis* species. The host range of most temperate phages isolated was restricted to one strain, in contradistinction to the sewage phages isolated[2].

Some phage suspensions appeared to contain mixtures of phages. This is being investigated. It is possible that if a greater variety of methods of induction and a wider range of indicator strains had been used more lysogenic *Proteus* strains would have been detected.

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Influence of Carotenoids on the Infra-Red Spectrum of Bacteriochlorophyll in *Chromatium*

Although it is well known that the infra-red maxima in the absorption spectrum of *Chromatium* exhibit considerable variability, the basis of this phenomenon has remained obscure. Wassink *et al.*[1] described in detail the variations which they observed in the infra-red spectrum of both the organisms and colloidal extracts. After considering several explanations, these authors took the view that all these infra-red maxima represent one pigment, namely, bacteriochlorophyll bound to different proteins. More recently, Duysens[2] also has claimed that each of the infra-red peaks represents bacteriochlorophyll; however, he has not tried to explain the existence of more than one peak. Work in this laboratory has led to a hypothesis of the ultra-structure of the bacterial chromatophore[3]. It was posulated from this model that the transfer of energy from carotenoids to bacteriochlorophyll has spatial requirements which are met only when the chromatophore is in a suitable environment and further that the complexity of the infra-red spectrum is related to the interaction between these two pigment systems. At this time both postulates have received experimental support. Variation in the concentration of inert solute in the suspension medium has pronounced effects upon the efficiency with which quanta absorbed by the carotenoids are used for photophosphorylation by isolated chromatophores[4]. The loss of the ability to transfer energy is also correlated with specific changes which appear in the infra-red spectrum. These changes which appear in the infra-red spectrum of isolated chromatophores are comparable to the differences which are observed in organisms with differing carotenoid content.

In Fig. 1 the changes in light absorption which accompany the 'uncoupling' between the carotenoids

and bacteriochlorophyll in the chromatophore are compared with the changes which occur when carotenoid defficiency is induced by diphenylamine treatment[5]. The infra-red spectrum of the isolated chromatophores in which the two pigments are 'coupled' is identical with the spectrum obtained *in vivo*; similarly, the activity of the carotenoids for photophosphorylation in such preparations is comparable to the activity *in vivo* for carbon dioxide fixation[4]. Both 'uncoupling' and carotenoid deficiency are associated with an increase in absorption in the 800 mμ maximum and a disproportionate decrease of the maxima at 850 and 890 mμ, respectively; in addition, the latter two peaks tend to shift to lower wave-lengths. A similar family of curves (Fig. 2) results with the spectra obtained *in vivo* from normal cultures which differ in carotenoid content. These changes are remarkably similar to the alterations produced by the interaction between certain dyes; for example, chrysophenine *G* and sky blue *FF*[6]. Although these observations are consistent with the view that bacteriochlorophyll contributes to the absorption in the infra-red, they indicate that the fine structure of the infra-red spectrum is determined by more subtle effects which are related to an interaction between bacteriochlorophyll and the carotenoids. An analysis and theoretical evaluation of this phenomenon will be presented in detail elsewhere.

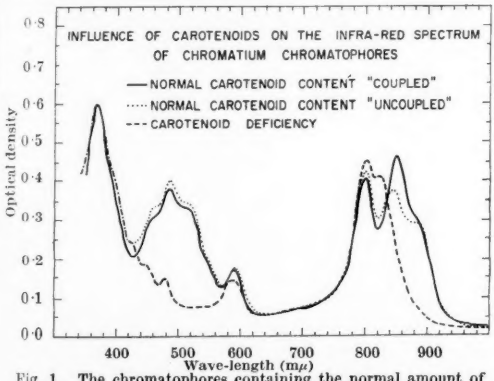
INFLUENCE OF CAROTENOIDS ON THE INFRA-RED SPECTRUM OF CHROMATIUM CHROMATOPHORES
NORMAL CAROTENOID CONTENT "COUPLED"
NORMAL CAROTENOID CONTENT "UNCOUPLED"
CAROTENOID DEFICIENCY
Optical density
Wave-length (mμ)

Fig. 1. The chromatophores containing the normal amount of carotenoids were prepared from a 48-hr.-old culture. The 'coupled' chromatophores were isolated and suspended in 0·5 *M* sucrose buffered to *p*H 7·8 in 0·1 *M tris*. The 'uncoupled' chromatophores were isolated and suspended in 0·4 *M* glucose at the same *p*H (ref. 3). The carotenoid deficient chromatophores were prepared in the sucrose medium from cells grown in the presence of diphenylamine (ref. 5). The spectra are compared using the Soret peak at 370 mμ as a reference

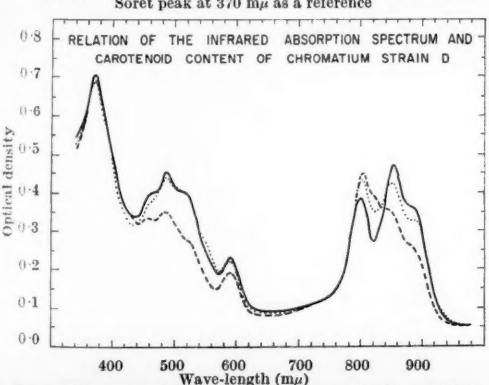
RELATION OF THE INFRARED ABSORPTION SPECTRUM AND CAROTENOID CONTENT OF CHROMATIUM STRAIN D
Optical density
Wave-length (mμ)

Fig. 2. The spectra above show the normal carotenoid variability found in cultures of differing ages. Opal glass was used to minimize scattering produced by the cells

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Release of Compounds containing Diaminopimelic Acid from *Vibrio metchnikovi* treated with Antibody and Complement

There is now much evidence supporting the suggestion that it is the peptide-amino sugar complex of the walls of Gram-negative bacteria which is responsible for the rigidity of the surface 'envelope'[1]. Loss of these components from the cell, either by direct enzymic digestion as with lysozyme, or by the metabolic disturbances brought about by penicillin action or diaminopimelic acid deprivation, results in a weakening of the wall and a transformation to spherical cells[1]. We have been interested in the possible similarity between the events responsible for immune bacteriolysis and those leading to the formation of spherical cells during lysozyme treatment and penicillin action.

When fresh guinea pig complement was added to cell suspensions of *Vibrio cholerae* and *V. metchnikovi* sensitized with their specific antisera, the cells became distorted and were eventually transformed into spherical cells of fairly uniform appearance. The conversion to spherical cells was virtually quantitative within 1–2 hr. at 37° C. after the addition of the complement. To determine whether any release of compounds containing diaminopimelic acid accompanied these morphological changes, thick suspensions of cells of *V. metchnikovi* were incubated with the appropriate amount of antibody and complement which effected the transformation to spherical cells on incubation for 2 hr. at 37°, and the supernatant fluids were examined for the presence of diaminopimelic acid. Control suspensions with antibody alone, complement alone and neither antibody nor complement were incubated under identical conditions; a small proportion of spherical transformations occurred in the control series. Cells were removed by centrifugation and the supernatant fluids were de-proteinized by the addition of trichloroacetic acid to a final concentration of 5 per cent w/v. The material soluble in trichloroacetic acid was extracted with ether to remove the acid, dialysed and then hydrolysed with *N* hydrochloric acid for 16 hr. at 105° C., and the diaminopimelic acid contents were estimated by the colorimetric method of Work[2] after separation on paper chromatograms using the solvent system of Rhuland *et al.* The amount of diaminopimelic acid released in the form of a non-dialysable compound soluble in trichloracetic acid, during treatment with the antibody-complement system is compared with the control series in Table 1. The cell wall of *V. metchnikovi* contained 0·5 per cent diaminopimelic acid, and if it is assumed that the wall accounts

Table 1. Effect of Antibody and Complement on the Release of Soluble Compounds containing Diaminopimelic Acid from *Vibrio metchnikovi*

Treatment	Diaminopimelic acid released from 200 mgm. dry-weight cells (μgm.)
Cells incubated with antibody and complement	104
Cells incubated with antibody alone	36
Cells incubated with complement alone	36
Cells alone	34

for 20 per cent of the weight of the cell, the amount of diaminopimelic acid released on treatment with antibody and complement would represent half the cell-wall diaminopimelic acid (if the wall accounted for a smaller fraction of the whole cell then of course the proportion of the wall diaminopimelic acid released will be greater).

These results make it clear that the morphological changes occurring during immune bacteriolysis with antibody and complement are accompanied by a release of soluble, non-dialysable components containing diaminopimelic acid. The cell constituents released are almost certainly derived from the bacterial cell wall. Thus, it is reasonable to conclude that the spherical transformation occurring during immune bacteriolysis (Pfeiffer's phenomenon) is explicable in terms of an enzymic (?) release of the cell-wall peptide which in the normal cell provides the wall with a rigid structural framework. Amano *et al.*[4] have suggested the possibility of enzymic disintegration of the cell wall playing some part in immune bacteriolysis and the results reported here contribute experimental evidence in general accord with this view. From light microscopic studies, Amano *et al.*[4] infer the complete disintegration of the wall by complement and antibody. However, our experience with isolated walls of *V. metchnikovi* incubated with complement and antibody indicated no appreciable lysis. Whether the complement acts enzymically or activates an enzyme system normally present in the cell cannot be said at the moment.

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Group and Type-specific Polysaccharides of Group *D* Streptococci

McCarty[1] showed that in group *A* streptococci the serologically group-specific polysaccharide (Lancefield's "*C* substance") is a major component of the bacterial cell wall and contains glucosamine and rhamnose. The type-specific proteins ("*M* substance") are located at the surface of the cell wall from which they may be detached by proteolytic digestion[2,3].

Recent work here has shown that in group *D* streptococci, which include the predominant intestinal streptococci of mammals and birds, a different situation exists. The results of this work are summarized in Table 1 from which it will be seen that

Table 1. Characteristics of Two Polysaccharides from Group *D* Streptococci

Probable location in Streptococcus	Serological specificity	Component sugars
Cell wall	Cell-specific	Hexosamine Rhamnose
Cell counts	Group-specific	Glucose

the cell-wall polysaccharides of group *D* are serologically type-specific instead of group-specific as in group *A*. Acid hydrolysates of these cell-wall polysaccharides from five different serological types of group *D* streptococci contained hexosamine (probably glucosamine), rhamnose and glucose.

The group-specific polysaccharide in group *D* appears to be situated deep within the streptococcus from which it may be extracted by shaking with glass beads in a Mickle disintegrator. A preparation made in this way from Lancefield's group *D* strain '*C*3' (*Str. durans*) was partially purified by high-speed centrifugation to remove most of the cell-wall material followed by digestion with proteinases and nucleases. The final product after dialysis contained approximately 20 per cent (w/v) total carbohydrate and 0·6 per cent hexosamine. In precipitin tests it reacted strongly with group-reactive antisera made against group *D* strains of heterologous type but reacted only weakly with homologous type-specific antiserum. From the same streptococcus a cell-wall preparation, serologically type-specific, contained approximately 10 per cent total carbohydrate and 8·0 per cent hexosamine. Allowing for contamination of the group preparation with residual cell-wall material estimated at between 5 and 10 per cent of the total serologically reactive carbohydrate, it may be inferred that the group-specific polysaccharide probably contained no hexosamine. Indeed, glucose was the only sugar found when acid hydrolysates of the group polysaccharide were submitted to paper chromatography. By contrast, hexosamine accounted for approximately 80 per cent of the cell-wall type-specific polysaccharide isolated from the same strain of streptococci.

Clearly, these results need confirmation with more highly purified material; but the present evidence suggests that the type-specific antigens in group *D* are the structural and chemical counterparts of the group-specific polysaccharide in group *A* streptococci. Although the evidence is not conclusive it seems likely that in group *D* streptococci the group-specific polysaccharide is situated deep within the bacterial cell. The difference in location and chemical constitution of the group-specific antigens in group *D* and group *A* may account for the greater difficulty generally experienced in making 'grouping' antiserum with group *D* streptococci and would help to explain Shattock's[4] observation that, for inducing the formation of group-specific antibodies in rabbits, a vaccine consisting of disrupted group *D* streptococci is more effective than one consisting of intact micro-organisms.

A more detailed account of this work will appear elsewhere. My thanks are due to Dr. R. C. Lancefield for cultures of her 4 'type' strains of group *D* streptococci and corresponding antisera. This work was supported in part by a grant from the Helen Hay Whitney Foundation, New York.

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